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STRUCTURAL STUDIES ON SOME LYCOPODIUM ALKALOIDS

by

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A B S T R A C T

The synthesis of a model compound for the 3,4-dihydro-2-pyridone system present in α-obscurine was undertaken. 3,4,5,6,7,8-Hexahydrocarbostyril was synthesized and its spectral properties investigated. These were found to be very similar to those of α-obscurine.

The minor alkaloids of L. clavatum Linn. were investigated and a total of 9 alkaloids was isolated. These substances were identified and the structure of the one new alkaloid isolated was established. Some chemical transformations pertaining to structural and stereochemical problems in this group are described.

The alkaloids of L. lucidulum Michx. were examined. The constitution and stereochemistry of alkaloid L. 20, previously described by Manske and Marion, was established.

CONTENTS

I N T R O D U C T I O N

In 1881, Bödeker (1) pointed out that Lycopodium complanatum L., a representative of the vascular cryptogams, contained an alkaloid which he called lycopodine. Little interest was shown in the alkaloids of Lycopodium until Manske and Marion investigated in the 1940's many other Lycopodium species and undertook a systematic separation of the alkaloidal material. They reported the isolation of 35 different alkaloids (2). It was found that lycopodine was present in all species studied except L. cernuum and L. saururus.

Little progress was made toward the elucidation of the structures of these alkaloids until 1956-57, when Wiesner and co-workers (3,4) showed that annotinine, the major alkaloid of L. annotinum, possessed structure I. This was later confirmed by an X-ray crystallographic study of annotinine bromohydrin (5).

Further progress in the structural studies was reported in the spring of 1960, just previous to the beginning of this work, when MacLean and Harrison (6) proposed structure II for lycopodine, Ayer and Iverach (7) proposed structure III for α-obscurine, structure IV for β-obscurine and structure V (8) for lycodine and Wiesner, Valenta and co-workers (9) proposed structure VI for selagine.

On the basis of these structures, Conroy (10) suggested

that the Lycopodium alkaloids may arise in the plant by the cyclization of two straight polyacetate chains as in VII. He suggested that the common intermediate VIII, from which the structures of the six previously mentioned alkaloids can be derived, could be formed by an aldol condensation between the C-7 carbonyl group and the C-12 methylene.

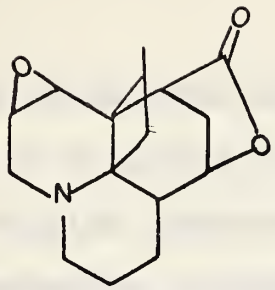
Lycopodine (II) could be obtained from the intermediate VIII by the following biogenetic pathway: Intermediate VIII undergoes an aldol condensation between C-8 and C-15, reduction of the double bond followed by a Mannich condensation of ammonia with C-13 and C-4 and then cyclization to the di-lactam IX which upon further reduction yields lycopodine (II).

To induce the intermediate VIII to give α-obscurine type skeleton, methylation of the nitrogen on C-13 (X) could prevent lactonization to the lycopodine skeleton, but permits the introduction of another nitrogen on C-5. After lactam closure and selective removal of the oxygen, the dihydropyridone system of α-obscurine (III) is obtained.

The biogenetic pathway for selagine (VI) closely parallels that of α-obscurine, except that the first nitrogen at C-13 lacks the methyl group and the C-9 carboxyl group is lost by decarboxylation of the β-keto acid. Unsaturations at C-11 and C-15 resulting from aldol condensations are still retained in selagine (VI).

Annotinine (I) could also arise from the intermediate VIII, especially if the C-8 methyl group is first oxidized to a carboxyl group, thus preventing the formation

CHART I



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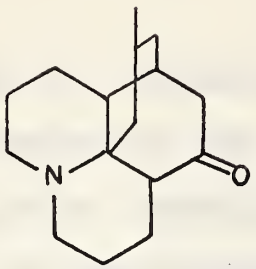

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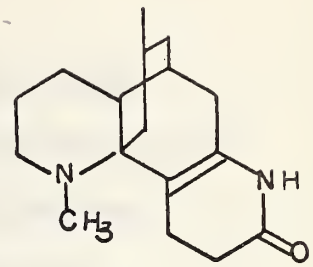

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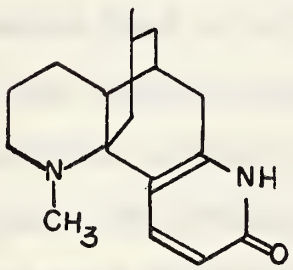

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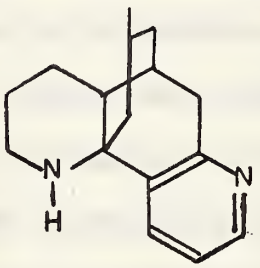

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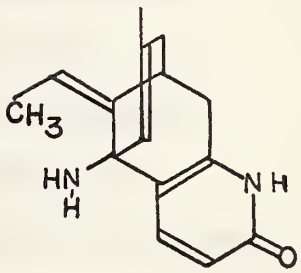

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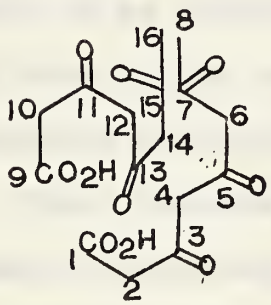

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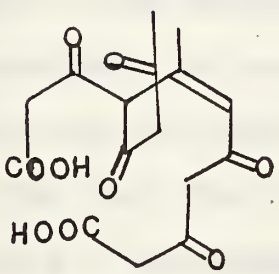

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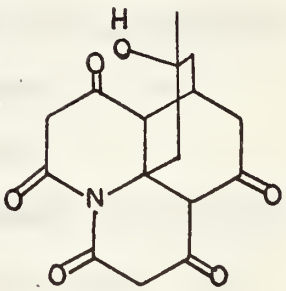

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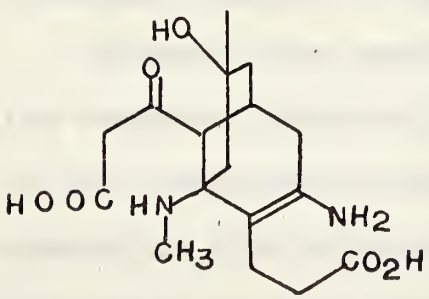

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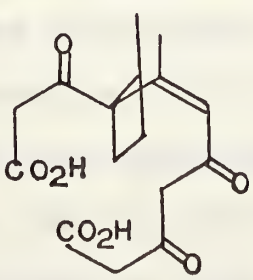

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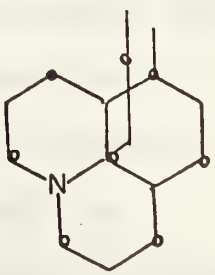


XII

of the lycopodine precursor IX. Closure can then take place at C-12 with the formation of the cyclobutane ring of XI. Mannich condensation as in lycopodine followed by lactamization, adjustment of the oxidation state and lactonization would give the naturally ocurring alkaloid (I).

On the basis of the Conroy biogenetic scheme, Wiesner (11) notes that a tricyclic intermediate such as XII, where the circled carbons denote the carboxylate units of the acetate, can only be a precursor of the annotinine skeleton. The precursor of lycopodine (II) cannot be tricyclic and the most advanced common skeleton to both lycopodine and annotinine must be bicyclic, that is, a skeleton of type XII cannot be an intermediate in the formation of the Lycopodium alkaloids, since any mechanisms for ring closure to the lycopodine skeleton would violate Bredt's rule.

This, coupled with the fact that lycopodine is the most commonly occurring alkaloid of Lycopodium, could mean that lycopodine is the central intermediate in the biogenetic formation schemes of Lycopodium alkaloids.

Wiesner also considers significant the similarity between the hemiketal form of annofoline (XIII) and annotinine (I). If the lycopodine skeleton was oxygenated at C-8 to give annofoline type alkaloids, the C-15/C-8 bond could be cleaved to give an intermediate of type XII where C-8 now is a carboxyl group, and could lead directly to the annotinine skeleton (I).

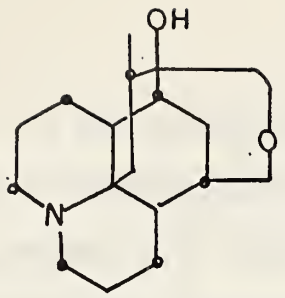
The above biogenetic pathways are convincing to a point. They, however, do not account for several observations.

First, one cannot say that methylation of a nitrogen atom attached to C-13 in the intermediate necessarily causes the molecule to adopt the obscurine-type skeleton since, as will be shown later, de-N-methyl α-obscurine (XIV) also occurs naturally as do both lycodine (V) and N-methyl-lycodine (XV) (12). This would seem to point to the methylation as a sporadic rather than a systematic step.

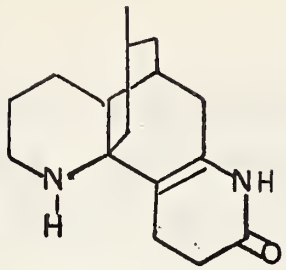
Also, the structures of alkaloids are now known which posses oxygen functions on carbons which are not derived from the carboxyl groups of the acetate units. These are lycodoline (XVI), whose structure was recently elucidated by Ayer and Iverach (13), flabelliformine (XVII), whose structure has been determined by MacLean and Curcumelli-Rostomo (14) and alkaloid L.20 (XVIII) whose structure will be discussed later.

Thirdly, the above mentioned biogenetic schemes ignore the fact that nicotine (XIX) is present in many Lycopodium species. It seems somewhat unlikely that the plant would have two unrelated alkaloid-forming enzyme systems, and it has been shown (15) that in Nicotinia species acetate is not a precursor of nicotine. It is also interesting to note that the skeleton of anabasine (XX) (another Nicotinia alkaloid) is present intact in the lycodine skeleton (see XX and XXI).

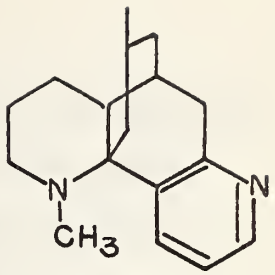
CHART II



XIII



XIV



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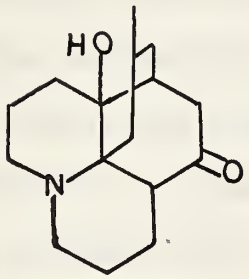
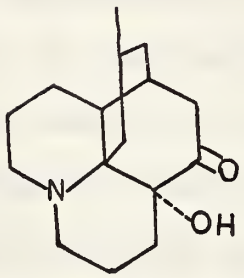
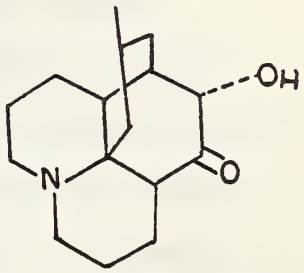


XVI



XVII



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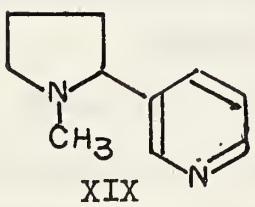

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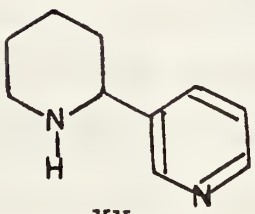
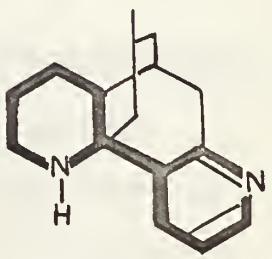
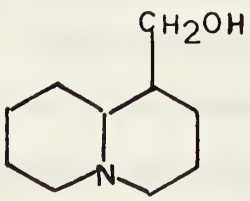


XX



XXI



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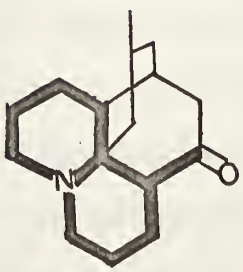
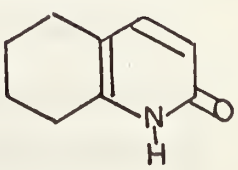


XXIII



XXIV

It has been shown that the saturated ring of anabasine (XX) is derived from lysine. The skeleton of the lupine alkaloids, e.g. lupinine (XXII), which are also derived from lysine, is present intact in the lycopodine-like Lycopodium alkaloids (see XXII and XXIII), and the Lycopodium alkaloids are invariably oxygenated at the same carbon as the lupine alkaloids. In the biosynthesis of nicotine, ornithine, the next lower homolog of lysine, is incorporated. Therefore, a scheme through the amino acids does not seem to be precluded and the Conroy acetate scheme must be viewed with some reservations until adequate feeding and tracer experiments have been carried out.

THE SYNTHESIS OF 3, 4, 5, 6, 7, 8 - HEXAHYDROCARBOSTYRIL A MODEL COMPOUND IN THE α-OBSCURINE SERIES

When this work was initiated, only the structure of annotinine was proven beyond doubt, and the structure of lycopodine the obscurines, lycodine and selagine were known with varying degrees of certainty.

Two alkaloids, which have been shown to have carbon skeletons different to that of lycopodine, but which as indicated earlier can be derived biogenetically from the same intermediate VIII, are α-obscurine and β-obscurine.

These minor alkaloids were separated by crystallization from L. annotinum L.(16), L. flabelliforme (17) and L. obscurum var. dendroideum (18), as obscurine, a single component.

By chromatography on alumina, Moore and Marion (19) resolved the obscurine into α-obscurine and β- obscurine.

α-Obscurine which analyzed for $C_{17}H_{26}ON_2$, was shown to posses a $>CHCH_3$ group, and a 3° nitrogen carrying a methyl group. It absorbed in the infrared (CCl_4) at 1700 cm^{-1} (m) and 1675 cm^{-1} (s). α-Obscurine showed a maximum in the ultraviolet at 255mμ (logϵ 3.73). The NMR spectrum showed peaks at 1.94τ (broad, due to N-H), 7.55τ ($N-CH_3$) and 9.14τ (doublet, $>CHCH_3$).

Dehydrogenation of α-obscurine with palladium and charcoal gave 7-methyl-quinoline and 6-methyl-α-pyridone accounting for 16 carbons of the molecule and suggesting that α-obscurine must contain a partially reduced α-pyridone ring. Also, α-obscurine could be easily transformed into β-obscurine by treatment with N-bromosuccinimide in carbon tetrachloride (20).

β-Obscurine analyzed for $C_{17}H_{24}ON_2$. Since α-obscurine could be easily converted by a dehydrogenating agent into β-obscurine, the latter must also possess $N-CH_3$ and $>CHCH_3$ groups. β-Obscurine showed absorption in the infrared (CCl_4) at 3385 cm^{-1} (N-H), 1659 cm^{-1} (N-CO-) and 1620 cm^{-1} and 1613 cm^{-1} (weak peaks). Maximum absorption in the ultraviolet occurred at 232mμ(logϵ 3.98) and 315mμ(logϵ 3.89). Both the infrared absorption and the ultraviolet are similar to that of 6-Me-α-pyridone. 6-Me-α-pyridone absorbs in the ultraviolet at 229mμ(logϵ 3.87) and 304mμ(logϵ 3.83) and in the infrared at 1662 cm^{-1} and 1651 $^{-1}$ (strong),

1628 cm^{-1} and 1613 $^{-1}$ (weak). The NMR spectrum of β-obscurine showed the following peaks: 2.21τ and 3.63τ (centers of doublets, J = 10 cps, ortho α-pyridone protons), 7.55τ ($N-CH_3$) and 9.14τ ($CHCH_3$). β-Obscurine was converted into α-obscurine by reduction with lithium-ammonia.

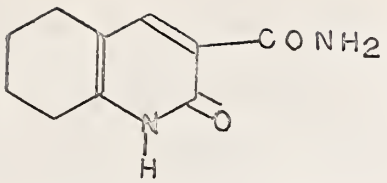
From the above data, it can be concluded that α-obscurine must possess a dihydropyridone ring in its skeleton.

With this information in hand and making the assumption that the obscurines might possibly arise from a precursor such as X, Ayer and Iverach proposed structures III and IV respectively for α-obscurine and β-obscurine. In order to more rigorously establish the presence of the 3,4-dihydro-2-pyridone system in α-obscurine, we set out to synthesize 3,4,5,6,7,8-hexahydrocarbostyril (XXVII) as a model of the B/C ring system of α-obscurine, since the literature contained no data on the ultraviolet and infrared characteristics of this system.

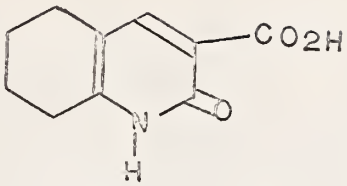
The first object in the synthesis of the model compound was 5,6,7,8-tetrahydrocarbostyril (XXIV) which would serve as a good model for the β-obscurine chromophoric system. The procedure of Dornaw and Neuse (20) was used.

Equimolar amounts of 2-hydroxymethylenecyclohexanone (21) and ethyl cyanoacetate were heated in an aqueous methanolic solution saturated with ammonia. From the diluted reaction mixture a solid, m.p. 320° C, separated. It absorbed in the

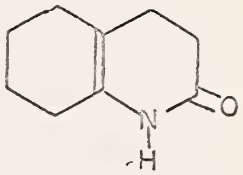
CHART III



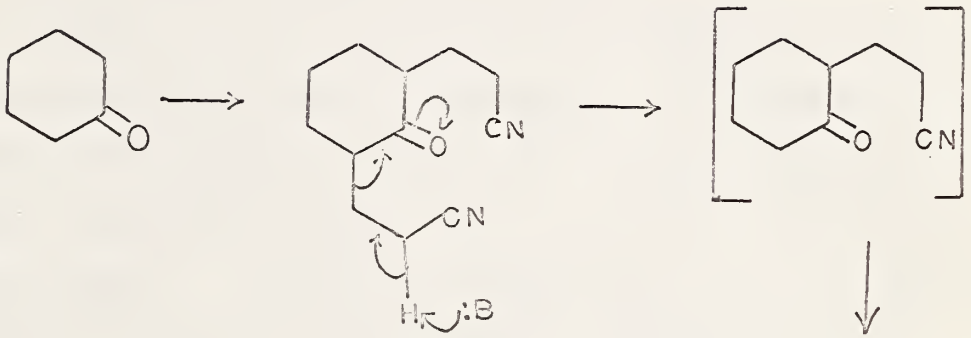
XXV

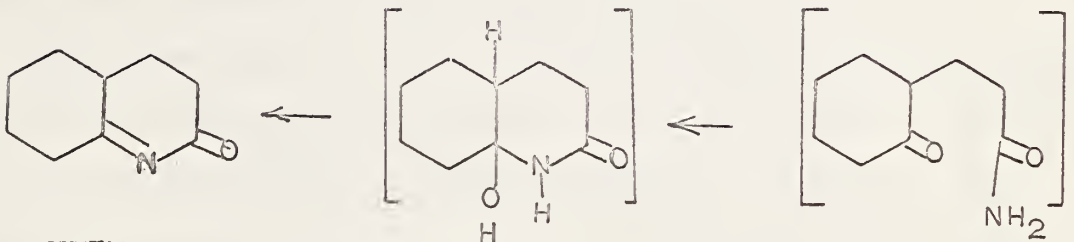


XXVI



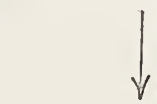
XXVII



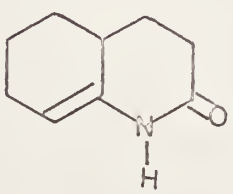


XXIX

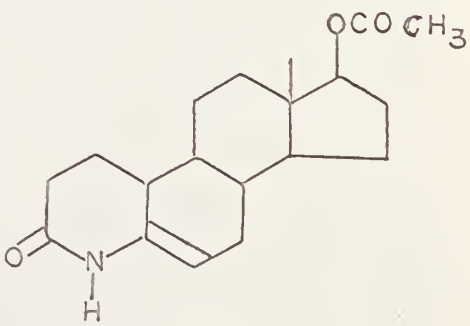
XXVIII



XXVII



XXX



XXXI

infrared (nujol) at 3290 cm^{-1} (s), 3130 cm^{-1}, 1673 cm^{-1} (s) with shoulders at 1630 cm^{-1} and 1610 cm^{-1}. The ultraviolet maxima were found at 240 mμ (log ε 3.88) and 340 mμ (log ε 4.01). The compound was 3-amido-5,6,7,8-tetrahydrocarbostyril XXV and in agreement with the structure, the NMR spectrum showed the presence of 1 vinylic hydrogen at 1.77τ.

The 3-amido-5,6,7,8-tetrahydroacarbostyril was hydrolyzed to the corresponding acid with 80% H_2SO_4. Infrared absorption of the acid XXVI occurred at 1695 cm^{-1} (-COOH), 2800-2500 cm^{-1} (broad band indicative of acid - OH), 1645 cm^{-1} (cyclic α-β unsaturated amide). Ultraviolet maxima were found at 235 mμ (log ε 3.85) and 312.5 mμ (log ε 3.79). The NMR spectrum showed the presence of one vinylic hydrogen at 1.69τ.

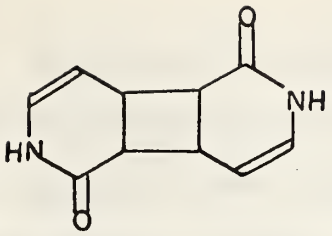
When 3-carboxy-5,6,7,8-tetrahydrocarbostyril was heated to 280° C at atmospheric pressure, decarboxylation occurred and 5,6,7,8-tetrahydrocarbostyril (XXIV) was obtained. The infrared spectrum (nujol) of 5,6,7,8-tetrahydrocarbostyril showed absorption at 3270 cm^{-1}, 1660 cm^{-1}. Maximum absorption in the ultraviolet occurred at 230 mμ (log ε 3.88) and 315 mμ (log ε 3.89). The NMR spectrum showed signals at 2.79τ (center of doublet with a separation of 9.0 cps) and at 3.67τ (center of doublet with a separation of 9.0 cps). All of the above spectral data corresponds very closely to that of β-obscurine.

Direct reduction of tetrahydrocarbostyril to the hexahydrocarbostyril with lithium in liquid ammonia (22) was not successful, and a direct method of preparing 3,4,5,6,7,8-hexahydro-

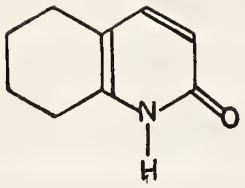
carbostyril (XXVII) was adopted. This method, previously reported by Campbell and Stevens (23) involved the treatment of 2,6-dicyanoethylcyclohexanone with methanolic KOH. The first step apparently is a reverse Michael reaction to give 2-cyanoethylcyclohexanone, which then is partially hydrolyzed to the corresponding amide, the nitrogen of the amide then adds to the carbonyl, giving XXVIII, which with a loss of a molecule of water and isomerization of the resulting double bond gives 3,4,5,6,7,8-hexahydrocarbostyril.

A priori, loss of water from the intermediate XXVIII could lead to either structure XXIX, XXX, or XXVII for the hexahydrocarbostyril thus obtained. Structure XXIX is eliminated by the infrared spectrum which shows N-H absorption at 3100 cm^{-1}, and the NMR spectrum which shows a broad, amide NH signal at 1.94 τ. Structure XXX is eliminated by the fact that the NMR spectrum does not show an olefinic proton and by the ultraviolet spectrum (see below). Both the analytical and the NMR data are consistent with structure XXVII. The ultraviolet spectrum of the 3,4,5,6,7,8-hexahydrocarbostyril was virtually superimposable on that of α-obscurine, thus proving the presence of the 3,4-dihydropyridone system in α-obscurine. It is interesting to note that the ultraviolet spectra of "homoannular enamides" such as 3,4,5,6,7,8 hexahydro carbostyril differ markedly from their "heteroannular" analogs, e.g. XXXI (24) Compounds of the latter type show a maximum in the ultraviolet at 234 - 235 mμ.

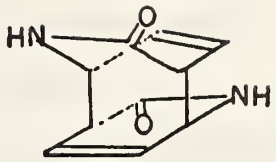
CHART IV



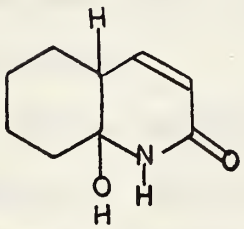
XXXII



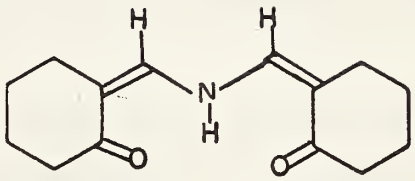
XXXV



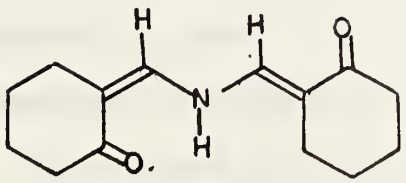
XXXIII



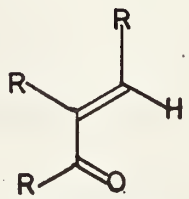
XXXIV



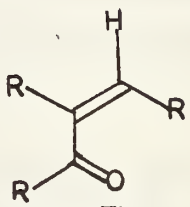
XXXVI



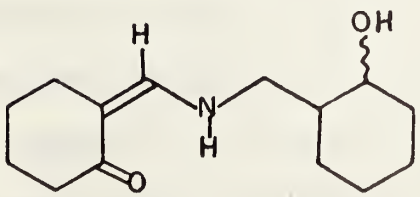
XXXVII



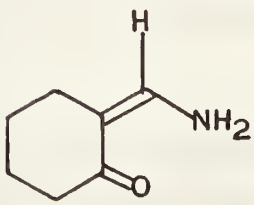
XXXVIII



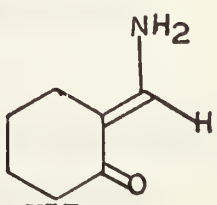
XXXIX



XL



XLA



XLB

Shortly after this work was completed, structure XXXII (25) was proposed for the photodimer of α-pyridone. It was reported that the photodimer showed no intense absorption above 220mμ, clearly inconsistent with the presence of two 3,4-dihyropyridone units. Further work in this laboratory (26) has shown that indeed the photodimer does not have structure XXXII and is instead XXXIII, a dimeric 3,6-dihydro-2-pyridone.

While surveying the literature for synthetic paths leading to the 5,6,7,8-tetrahydrocarbostyril system, we came across a synthesis by Sen Gupta (27). This method was almost identical with that of Dornaw and Neuse already mentioned, except that Sen Gupta's procedure was actually a Knoevenagel condensation, where cyanoacetamide was used instead of ethyl cyanoacetate in the presence of dimethylamine.

The product of this reaction, Sen Gupta stated, was a mixture of two compounds, whose structural formulas differed form one another by a molecule of water. The compound containing the additional water molecule, he postulated, was the intermediate XXXIV, which had failed to dehydrate to the tetrahydrocarbostyril system.

However, we suspected that under the reaction conditions the cyano group underwent hydrolysis to the amide thereby acquiring a molecule of water, to establish this, the Sen Gupta synthesis was carried out.

The product obtained was separated into the two components, on the basis of its partial solubility in chloroform. The

chloroform soluble portion absorbed in the infrared (nujol) at 3340 cm^{-1}, 3140 cm^{-1}, 2250 cm^{-1} (-CN), 1647 cm^{-1}. Therefore, the chloroform soluble compound was the 3-cyano-5,6,7,8-tetrahydrocarbostyril (XXXV). The chloroform insoluble portion absorbed in the infrared at 3300 cm^{-1}, 3130 cm^{-1}, 1673 cm^{-1} with a shoulder at 1612 cm^{-1}. This spectrum was identical with that of 3-amido-5,6,7,8-tetrahydrocarbostyril (XXV), thereby establishing the identity of Sen Gupta's hydrated compound.

During this work, it was observed that when 2-hydroxymethylenecyclohexanone was treated with ammonia in aqueous methanol, the solution became bright yellow and when the solution was diluted with water, yellow crystals, mp. 155°C, separated. Analysis and molecular weight determination indicated the formula $C_{14}H_{19}O_2N$ for this compound.

The infrared spectrum (nujol) showed absorption at 1661cm^{-1} and 1644 cm^{-1}, indicative of a β-amino-α-β-unsaturated ketone (28). The ultraviolet spectrum showed maxima at 383 $m\mu$ ($\log\epsilon$ 4.46), 283 $m\mu$ ($\log\epsilon$ 3.42) and 253 $m\mu$ ($\log\epsilon$ 3.47). The long wavelength absorption in the ultraviolet indicated a system of extended conjugation and together with the infrared data and the molecular formula suggested structure XXXVI for the yellow compound.

The NMR spectrum, however, did not agree with this. In structure XXXVI the olefinic protons are equivalent. The NMR spectrum of the yellow compound showed two different olefinic

proton signals, one at 2.58τ (doublet, J = 13 cps) and one at 3.26τ (doublet, J = 11 cps) as well as peaks at 7.56τ (8 protons, methylenes adjacent to unsaturation) and at 8.18τ (8 protons, saturated methylenes). A broad peak at -1.6τ was assigned to the proton on nitrogen. This data is consistent with XXXVII, the geometrical isomer of XXXVI.

It is known that the β-hydrogens in the cisoid α-β unsaturated carbonyl system XXXVIII are more deshielded (29) than in the transoid α-β unsaturated system XXXIX. Therefore, the signal at 2.58τ can be attributed to the cisoid arrangement of the vinylic hydrogen with respect to the carbonyl group, and the 3.26τ value to the transoid. That the doublets arise from the coupling of the vinylic hydrogens with the N-H was shown in the following way. A solution of the yellow compound in $CDCl_3$ was shaken with D_2O. The NMR spectrum then showed singlets at 2.60τ and 3.25τ and did not show any absorption in -1.6τ region, confirming the presence of N-D. The collapse of the doublets to singlets would be expected upon replacement of the hydrogen on nitrogen with deuterium.

Through the spectral data, the structure and the stereochemistry of the unknown compound were estblished.

An attempt was made to prepare this yellow compound by treating 2-hydroxymethylenecyclohexanone in benzene with ammonia (30). The product, however, was not the yellow compound but 2-aminomethylenecyclohexanone. The infrared spectrum showed absorption (nujol) at 3340 cm^{-1}, 3273 cm^{-1},

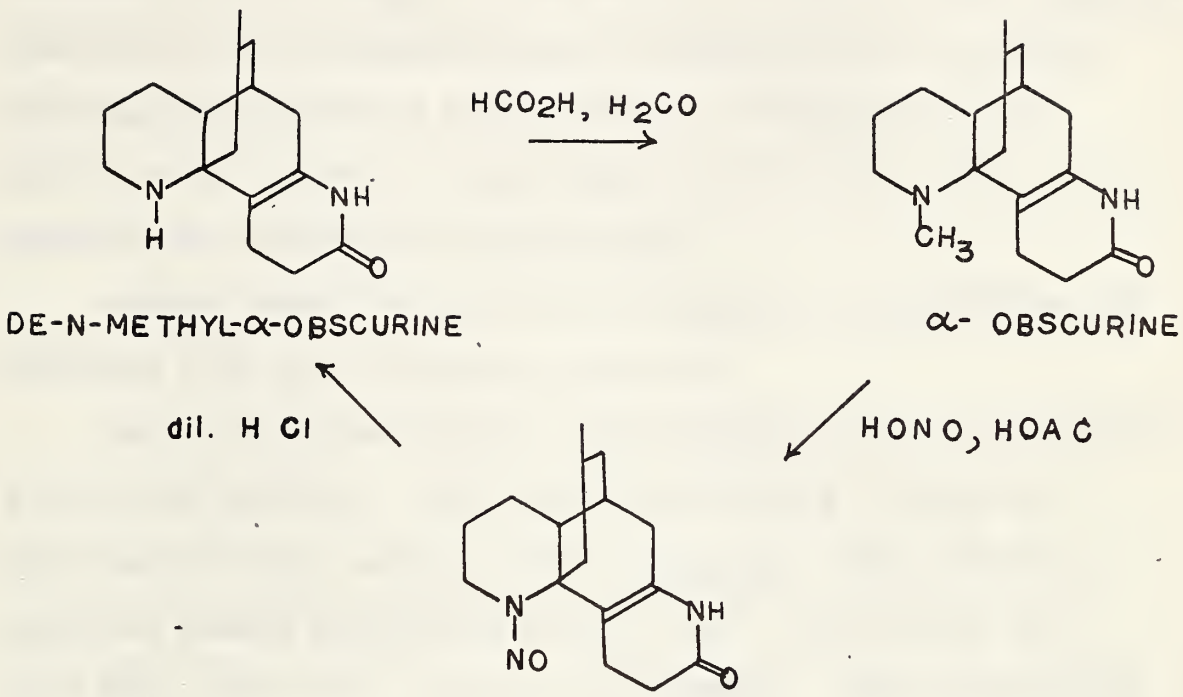
3173 cm^{-1} (NH_2), 1655 cm^{-1} (β-amino -α-βunsaturated ketone). The ultraviolet maximum was at 313$m\mu$(log ϵ 4.29), which is in the region reported for such unsubsitituted enamines (30).

The NMR spectrum indicated that the product consisted of two geometric isomers (XLA and XLB), where the vinylic hydrogens were present in cis and trans arrangement with respect to the carbonyl group. Two triplets were found at 2.36τ (cisoid) and at 3.29τ (transoid) having intensities of 1 and 3 respectively. That the triplets arose from the coupling of cis and trans vinylic hydrogens with the -NH_2 was shown as in the case above. A solution of the 2-aminomethylenecyclohexanone in $CDCl_3$ was shaken with D_2O. The collapse of the triplets did take place and singlets were now found at 2.35τ and 3.30τ respectively.

An attempt to separate the product into the two geometric isomers by chromatography on alumina was not successful. Each fraction obtained contained the same proportion of cis-2-aminomethylenecyclohexanone and trans-2-aminomethylenecyclohexanone.

Since the yellow compound failed to form in the absence of water, it was concluded that both 2-aminomethylenecyclohexanone and 2-hydroxymethylenecyclohexanone were necessary for this particular reaction to take place and that in the above reaction the 2-hydroxymethylenecyclohexanone is converted almost completely to the amino form. The mechanism for the formation of the yellow

CHART V


HCO2H, H2CO
DE-N-METHYL-α-OBSCURINE
α- OBSCURINE
dil. H Cl
HONO, HOAc
NH
N
H
CH3
NO
O

compound must involve addition of the nitrogen to the carbonyl of the aldehyde form of 2-hydroxymethylenecyclohexanone with subsequent elimination of water. When equimolar quantities of 2-aminomethylenecyclohexanone and 2-hydroxymethylenecyclohexanone were reacted in benzene and the water formed removed by azeotropic distillation, the yellow compound was produced in good yield.

Further prcof of the yellow compound's composition was obtained from the following reactions.

The yellow compound was hydrogenated in the presence of a platinum catalyst. The compound absorbed 2 moles of hydrogen giving a white solid, $C_{14}H_{23}O_2N$. The infrared spectrum showed absorption at 3380 cm^{-1} (-OH), 3250 cm^{-1}, 3100 (NH), 1660 cm^{-1} (due to the enamine). The ultraviolet maximum was at 328 mμ (log ε 4.35).

Manson and co-workers (30) found that ultraviolet spectrum of enamines undergoes a bathochromine shift upon substitution of the hydrogens on the nitrogen with alkyl groups.

On the basis of the infrared and ultraviolet data, it can be concluded that the yellow compound underwent partial hydrogenation with reduction of one of the α-β unsaturated ketone systems and the product is simply a monosubstituted enamine as substantiated by the ultra-violet spectrum. It should be noticed here that 2-aminomethylenecyclohexanone absorbs in the ultraviolet at 313 mμ, but the

monosubstituted β-amino-α β unsaturated ketone system obtained above does exhibit the bathochromic shift and absorbs at 328mμ. The NMR spectrum showed signals at 3.33τ (doublet, J = 12 cps). This indicates that the cisoid system in the unknown compound was hydrogenated, since the doublet attributed to this system on the basis of the deshielding information has disappeared, and hence structure XL is probably the correct representation of the partially reduced compound.

When the yellow compound was reduced with sodium borohydride, a completely saturated product was obtained. The infrared spectrum ($CHCl_3$) showed a small peak at 3620 cm^{-1} and a deep, broad band at 3350 cm^{-1} but no carbonyl absorption. A study of the hydroxyl absorption in CCl_4 at various concentrations indicated much intramolecular hydrogen bonding, and the NMR spectrum showed the absence of olefinic protons.

Further proof of the structure was obtained by basic hydrolysis of the yellow compound. When the yellow compound was refluxed in methanolic NaOH, ammonia was evolved and cyclohexanone was separated from the reaction mixture and characterized as the 2,4 dinitropheny. hydrazone. Formic acid should also have been produced, but no attempt was made to recover it.

THE MINOR ALKALOIDS OF L. CLAVATUM

The alkaloids of L. Clavatum Linn. were first examined by Achmatowicz and Uzieblo (31). These workers, using L. Clavatum Linn. collected in Poland isolated three alkaloids, lycopodine, clavatine, $C_{16}H_{25}O_2N$, mp. 212-213°C, and clavatoxine, $C_{17}H_{27}O_2N$, mp. 185-6°C. Later Manske and Marion (32) examined L. Clavatum Linn. native to Canada and reported the isolation of lycopodine, a substance which they called alkaloid L. 13 but which has since been shown to be identical to lycopodine, as well as two other poorly defined new alkaloids, designated as L.18 and L.19. Alkaloid L.18 was isolated only as the picrate and appeared to have an empirical formula $C_{11}H_{19}ON$, but in view of the fact that most of the Lycopodium alkaloids have at least 16 carbon atoms, this must be viewed with some scepticism.. Alkaloid L.19, mp. 231°C was isolated in very small quantity and was not analyzed. In view of the fact that we have isolated clavolonine (L.34, see below) mp. 223-34°C, from L.clavatum it is not inconceivable that alkaloid L.19 is in fact identical to clavolonine. Manske and Marion were unable to isolate clavatine and clavatoxine, the alkaloids reported by Achmatowicz and Uzieblo.

Since L. clavatum was used in these laboratories as a source of lycopodine during structural studies on the latter, we have had occasion to reexamine the minor alkaloids of this species. Elution chromatography over alumina followed by (fractional) crystallization of the fractions thus obtained

lead to the isolation of the following alkaloids:

i) Lycopodine (I), $C_{16}H_{25}ON$, mp. 116°C, the most commonly occuring alkaloid of the Lycopodium species

ii) Lycodine, $C_{16}H_{22}N_2$, mp. 118°C, which was first isolated by Anet and Eves (33). The structure V was determined by Ayer and Iverach (8)

iii) Dihydrolycopodine, $C_{16}H_{27}ON$, mp. 167°C, first isolated by Manske and Marion from L. complanatum (34). It is also identical to the product of the lithium aluminum hydride reduction of lycopodine (35).

iv) Clavolonine (alkaloid L.34), $C_{16}H_{25}O_2N$, mp. 241°C, which was first isolated by Manske and Marion (36) from L. densum L. found in New Zealand. The structure XLI was determined by Burnell and Taylor (37).

v) A substance, $C_{16}H_{25}O_2N$, mp. 214°C, which was isolated from L. flabelliforme by MacLean and Curcumelli - Rostomo (14). The substance isolated by us was identical with that separated by MacLean as shown by an undepressed mixed melting point and superimposable infrared spectra. The structure of "flabelliformine" has been recently determined by the above mentioned workers. The physical constants of this alkaloid correspond closely with those reported for clavatine (31), but a direct comparison of the two compounds has not been possible (38)

vi) A substance, mp. 208°C, which analyzed for $C_{32}H_{52}N_2O_3$. The fact that the empirical formula possessed 32 carbon atoms suggested that the 208° compound was either a "dimeric" type Lycopodium alkaloid or a 1:1 molecular complex of two different alkaloids. Chromatography over alumina did not effect any separation and the compound showed a single spot on paper chromatography. However, oxidation of the 208° compound with Sarett's reagent gave a new substance, $C_{32}H_{50}N_2O_3$, mp. 165°C, which showed peaks at 1710 cm^{-1} and 1695 cm^{-1} (the 208° compound shows only one carboxyl peak at 1707 cm^{-1}) as well as hydroxyl absorption in the infrared. Chromatography of the 165° compound over alumina separated it into two compounds, lycopodine, mp. 116°C, (eluted with benzene) and flabelliformine, mp. 214°C, (eluted with benzene-ether). Thus it appeared that the 208° compound might be a molecular complex of dihydrolycopodine and flabelliformine and that the former was oxidized to lycopodine. Flabelliformine is stable to Sarett oxidation. This was shown to be the case, since combination of equimolar quantities of dihydrolycopodine and flabelliformine (35) gave the 208° molecular complex, identical in all respects to that isolated from the plant.

vii) A substance, mp. 180°C, analyzed for $C_{16}H_{25}O_2N$. The infrared spectrum showed absorption at 3325 cm^{-1}, 1712 cm^{-1}, 1696 cm^{-1} and 957 cm^{-1} (this band also appears in the infrared spectrum of lycodoline). Because two carbonyl peaks were present in the infrared spectrum and on the basis of the analysis, the substance was thought to be a 1:1 complex of two $C_{16}H_{25}O_2N$ alkaloids.

Chromatography over alumina separated the complex into flabelliformine and lycodoline (alkaloid L.8). Also two spots were obtained on paper chromatoraphy of the complex with R_f values of .52 and .76. These were identical to those of lycodoline and flabelliformine, respectively.

Flabelliformine exhibits a great tendency to complex with other alkaloids, as indicated by the three molecular complexes described here.

viii) A substance, $C_{16}H_{24}ON_2$, mp. 268-71°C, absorbing in the infrared (nujol) 3250 cm^{-1} (N-H), 1699 cm^{-1} (shoulder), 1678 cm^{-1}, and 1644 cm^{-1}. The ultraviolet spectrum showed a peak at 255 mμ. On the basis of the spectral data which was very similar to that of α-obscurine, the compound was suspected of being de-N-methyl-α- obscurine (see Chart V), which had not previously been isolated from natural sources.

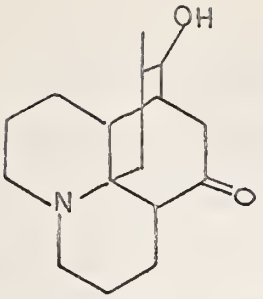
Methylation of the substance with formic acid and

formaldehyde gave a compound which was identical in all respects to α-obscurine. The infrared spectrum of the methylation product was superimposable on that of α-obscurine, and a mixed m.p. of the methylation product and authentic α-obscurine showed no depression.

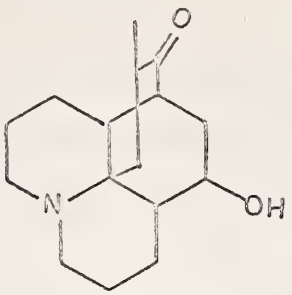
The infrared spectrum of this alkaloid, de-N-methyl-α-obscurine, was identical with that of the de-N-methyl-α-obscurine prepared by treatment of α-obscurine with nitrous acid (39) followed by hydrolysis of the resulting N-nitroso compound. The reactions are summarized on Chart V.

ix) A small amount of α-obscurine was also isolated. The presence of these two substances, α-obscurine and de-N-methyl-α-obscurine, in the same Lycopodium species is of biogenetic interest. Conroy's biogenetic scheme (10) assumes that the formation of the obscurine skeleton from the intermediate X , the precursor of all Lycopodium structural types, is induced by the methylation of the nitrogen on C-13. However, the presence of both α-obscurine and de-N-methyl-α-obscurine in the same plant makes this hypothesis doubtful, unless the compound is first methylated in the plant, and later undergoes demethylation under specific enzymatic conditions.

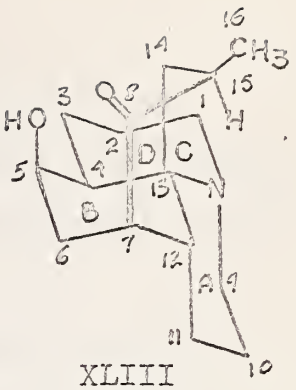
CHART VI



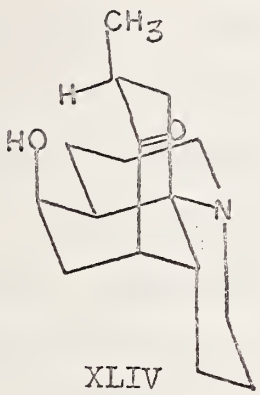
XLI



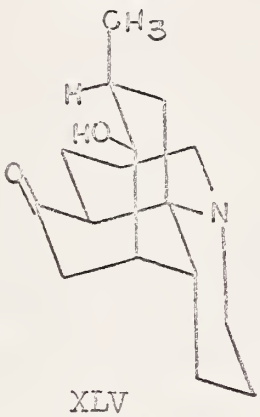
XLII



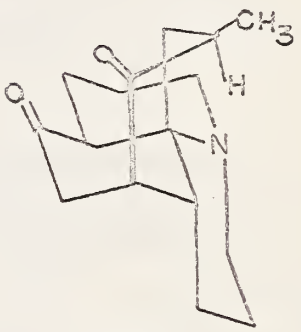
XLIII



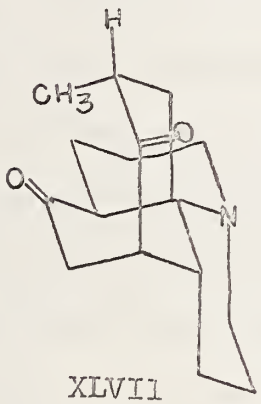
XLIV



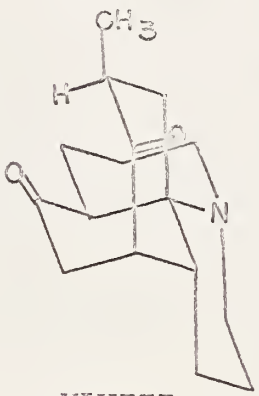
XLV



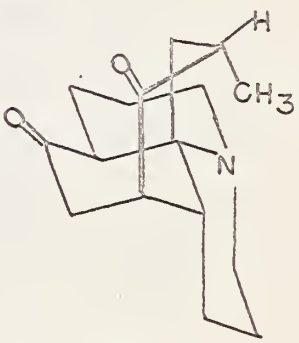
XLVI



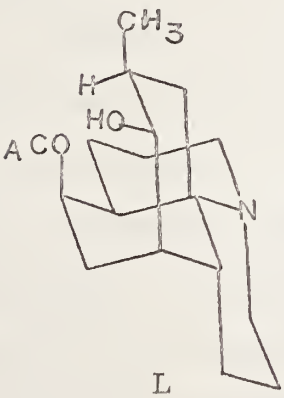
XLVII



XLVIII

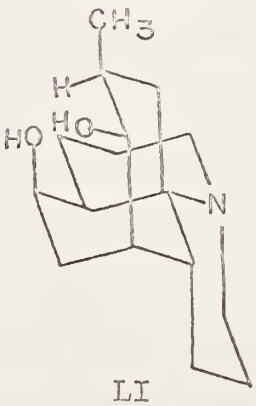


XLIX



L

$\xrightarrow{OH^-}$



LI

$\xrightarrow{CrO_3}$ XLI $\xrightarrow{CrO_3}$ XLVIII

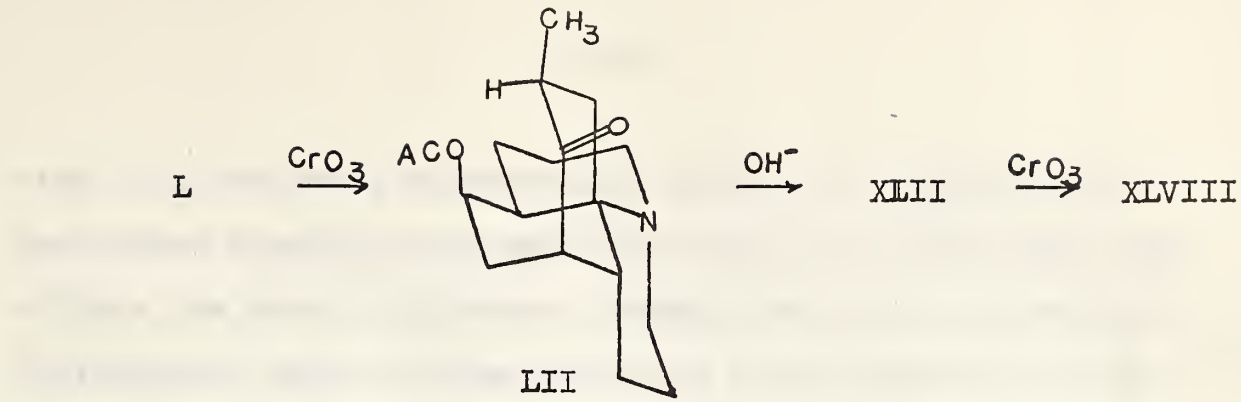
With the completion of the identification of these substances, it was hoped that some of them might prove useful in connection with other structural studies in progress in our laboratory.

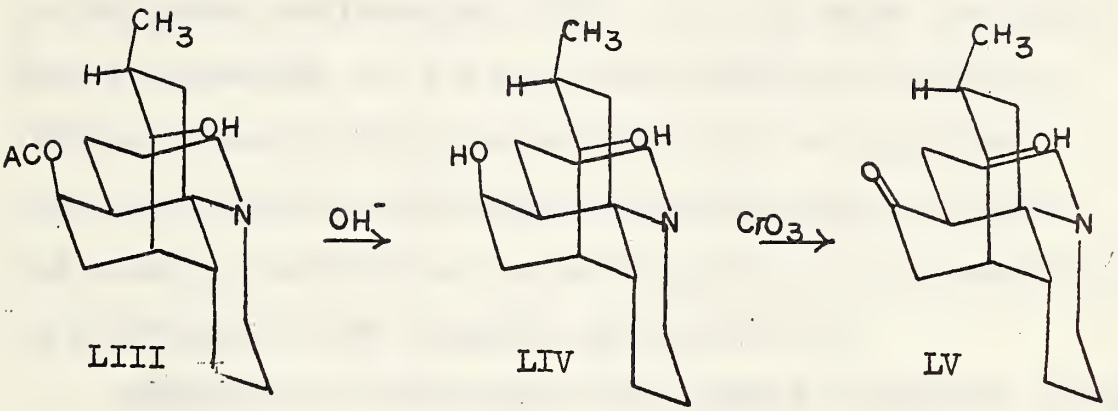
At this time work was in progress on three $C_{16}H_{25}O_2N$ alkaloids of then unknown structure. These were lycodoline, flabelliformine and a hydroxy ketone obtained from lycoclavine, a hydroxy acetate of then unknown structure.

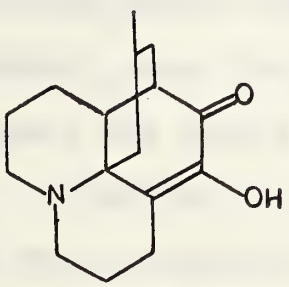
The structures of two other $C_{16}H_{25}O_2N$ alkaloids were then known. These were clavolonine (isolated above) and annofoline. Clavolonine had been shown to have structure XL (37). Annofoline was isolated by F.A.L. Anet and N. H. Khan (40) from lycopodium annotinum (40) and was shown to have structure XLII (41).

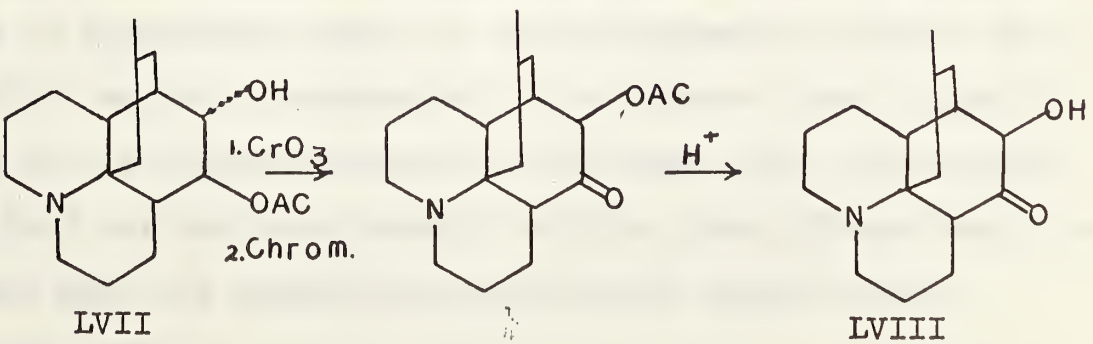
At the time it was thought that one or more of the three alkaloids of unknown structure mentioned above might possess the oxygen functions in the same positions as annofoline and clavolonine and simply be epimeric with them at one of the oxygenated carbons.

Synthesis of the diketones from annofoline and clavolonine would not only provide a simple method of checking this possibility but would also afford us an opportunity to examine the interesting chair-boat relationships of the ring D in these alkaloids. It has been shown (41) that ring D in annofoline exists in the boat conformation XLIII, with the methyl group in the boat equatorial position. Since annofoline is stable to base, this means that conformation XLIII is preferred over conformation XLV, which has the methyl group equatorial but


L
CrO3
ACO
CH3
H
O
N
LII
OH⁻
XLII
CrO3
XLVIII


CH3
H
ACO
OH
N
LIII
OH⁻
HO
LIV
CrO3
O
LV


O
N
OH
LVI


OH
1. CrO3
OAC
2. Chrom.
N
LVII
OAC
O
H+
OH
LVIII

ring D in the chair conformation, that is, the very serious non-bonded interaction between C-5 and C-15 in XLIV more than offsets the energy difference between the chair and the boat. Clavolonine, which is also stable to base, exists with ring D in the chair conformation (XLV). In this case the methyl group cannot epimerize, so the boat conformation is highly unfavored since it would necessarily involve a serious bowsprit-flagpole methyl-hydrogen interaction. Also in the case of clavolonine the serious C-5, C-15 interaction is minimized by the trigonal nature of C-5.

Oxidation of annofoline would afford a diketone which could have either conformation XLVI or XLVII. Examination of Drieding models suggests that conformation XLVI would be favored, since in XLII there are serious non-bonded interactions between the methyl group and ring B.

Oxidation of clavolonine would afford the diketone XLVIII. The boat conformation XLIX would undoubtedly be highly unfavored in this case (bowsprit-flagpole interaction, no serious C-5, C-15 interaction in the chair). Our interest lay in determining which of the two epimeric ketones (XLVI or XLVIII) would be thermodynamically favored, that is, whether the C-5, C-15 interaction in XLVIII was still large enough so that the boat conformation of ring D was favored over the chair when the methyl group was free to assume either configuration.

The clavolonine used for the oxidation studies was obtained from L. clavatum and when this was exhausted, it was prepared from fawcettiine (L) by hydrolysis to deacetylfawcettiine (LI) which on gentle oxidation gave clavolonine. Oxidation of clavolonine with either chromium trioxide-pyridine or chromium trioxide in acetic acid yielded, after chromatography, the corresponding diketone which showed carboxyl absorption at 1710 cm^{-1} and no hydroxyl bands in the infrared. The diketone could not be crystallized but was characterized as its crystalline methiodide.

Annofoline was prepared from fawcettiine (L) by Sarett oxidation to oxofawcettiine (LII) which was then hydrolyzed and epimerized to annofoline. Oxidation of annofoline with either chromic acid-acetic acid or chromium trioxide-pyridine led to the same diketone as that obtained from clavolonine. The identity of the diketones was established by comparison of their infrared spectra and by comparison of the infrared spectra, melting points and mixed melting points of their methiodides. It was now necessary to determine whether the diketone was actually the clavolonine diketone XLVIII or the annofoline diketone XLVII. Reduction of the diketone with lithium aluminum hydride afforded deacetylfawcettiine (Ll) in which the configuration of the methyl group is known, and hence the diketone must have structure XLVIII. Since the configuration of the methyl group in annofoline is fairly well established the conclusion must be drawn that the diketone XLVI initially

produced on oxidation of annofoline is much less stable than the diketone XLVIII and that under the conditions of the oxidation (i.e. in pyridine or acetic acid plus chromic acid) is rapidly epimerized to the diketone XLVIII. This finding clearly indicates that when C-5 becomes trigonal, thus relieving the C-5, C-15 interaction, the boat conformation is no longer favoured over the chair.

In connection with this work, the oxidation of deacetyllofoline (LIV) obtained by hydrolysis of lofoline (LIII), was also investigated. It possessed the same substituents on the same carbons as fawcettiine except that the hydroxyl group on C-8 is axial instead of equatorial.

Oxidation of this diol with Sarett reagent gave a colourless oil which crystallized from acetone. The compound analyzed for $C_{16}H_{25}O_2N$ and showed peaks in the infrared at 3185 cm^{-1} (-OH), 1710 cm^{-1} (six ring ketone) and 1420 cm^{-1} ($-CH_2-$adjacent to the ketone), and hence must be LV, which is epimeric with clavalonine at C-8.

Separation and characterization of "epiclavolonine" was prompted by the possibilty of its similarity to one of the known ketols, which was one of the basis of this phase of work. However, it proved to be quite different from all of these. It was also found that the diketone from clavolonine was different from that obtained in the lycoclavine series.

THE ALKALOIDS OF LYCOPODIUM LUCIDULUM. THE STRUCTURE OF ALKALOID L. 20

The Lycopodium alkaloids of known structure (excluding nicotine) fall into three groups: A, the lycopodine group, compounds possessing the lycopodine skeleton (II) and having, in general, 16 carbons and one nitrogen (exceptions to the 16 carbon rule are O-acetyl derivatives of the 16 carbon compounds); B, the dinitrogenous alkaloids such as selagine (VI),lycodine (V) and the obscurines (III, IV), which contain, respectively, fifteen, sixteen and seventeen carbon atoms, but which may be regarded biogenetically very similar; C, annotinine (I), the only member of the third group. Recently, interest in these laboratories has turned to a study of two compounds which do not fall into either of the above groups. These are the alkaloids annopodine, $C_{17}H_{25}O_3N$, first isolated by G.G.Iverach from L. annotinum, and a compound isomeric with annopodine called alkaloid A-2 isolated independently in these laboratories and at the University of New Brunswick.

Three other Lycopodium alkaloids containing seventeen carbons and one nitrogen have been reported in the literature. These are alkaloid L. 28, $C_{17}H_{27}O_2N$; isolated from L. acrifolium Fern. by Manske and Marion (42), clavotoxine, C H O N, reported by Achmatowicz and Uzieblo (31) and mentioned previously, and alkaloid L. 20, $C_{17}H_{27}O_2N$, isolated by Manske

and Marion (43). Since L. lucidulum was readily available to us, we decided to investigate the structure of L.20 in conjunction with the study of the other C_{17} compounds.

L. Lucidulum Michx. was soxhlet extracted with methanol and the crude alkaloid material (0 9% by weight of the dried plant) was separated into strongly and weakly basic alkaloids by dissolving the crude alkaloids in dilute acid, adjusting the pH to about 8 and extracting the weak bases with chloroform. The aqueous solution was then made strongly basic with with NH_4OH and extracted again with chloroform.

The weakly basic fraction was submitted to a counter current distribution between chloroform and buffer of pH 6. The weak bases were thus separated into lycopodine and an oily unknown alkaloid.

The infrared spectrum of the unknown alkaloid showed absorption at 1650 cm^{-1} in carbon tetrachloride and at 1628 cm^{-1} in chloroform suggesting the presence of an amide linkage.

Neither the unknown alkaloid nor its various derivatives such as the picrate, perchlorate or picrolonate were obtained in crystalline form. On standing, the alkaloid hardened to a varnish which decolorized progressively with time.

Analysis and molecular weight determinations on the non-crystalline material, purified by distillation, indicated a molecular formula $C_{26}H_{38}O_2N_2$, but since the homogeneity of this substance has not been established, this may well prove to be incorrect. This substance has not been investigated further.

Crystallization of the strong bases from methanol yielded alkaloid L.20, identical in all respects with a sample kindly provided by Dr. L. Marion. A total of 0.3 gms. was obtained from 24 lbs. of plant material. Chromatography of the mother liquors from this crystallization yielded lycopodine and lycodoline (alkaloid L.8) as the only crystalline materials. Lycodoline had not previously been isolated from L. lucidulum.

THE STRUCTURE OF ALKALOID L.20.

Since our interest in alkaloid L.20 stemmed from the report that it was a 17 carbon alkaloid, it was somewhat of a disappointment to discover that it was in fact a 16 carbon compound, our results fitting best the molecular formula $C_{16}H_{25}O_2N$. The infrared spectrum (nujol mull) showed absorption at 1722 cm^{-1} (unstrained ketone or aldehyde) and bands at 3050 cm^{-1} (n) and 2800 - 2500 cm^{-1} (indicative of an extensively intermolecularly hydrogen-bonded hydroxyl group). In chloroform solution, L.20 shows a peak at 3620 cm^{-1} (non-hydrogen bonded hydroxyl and 1710 cm^{-1} . The ultraviolet spectrum showed a peak at 296 $m\mu$ ($\log \epsilon$ 1.85). Ketones in six membered rings show maxima at 280 - 290 $m\mu$, lycopodine (II) for example, absorbs at 285 $m\mu$. It is known (44) that an axial hydroxyl group α to a ketone causes a bathochromic shift of 10-20 $m\mu$ in the ketone $\eta \rightarrow \pi^*$ absorbtion band. It seemed possible, then, that alkaloid L.20 was an α-(axial) ketol. Conformation of this was obtained from the optical rotatory dispersion spectrum which showed a

positive Cotton effect with the extrema occurring at 322.5 mμ ($[\alpha]^{MeOH}_{322.5}$ +1500) and 281 mμ ($[\alpha]^{MeOH}_{281}$ -3400).

Normally six membered ketones exhibit their first extrema at 300 ± 5 mμ as demonstrated by lycopodine, whose rotatory dispersion extrema occur at 307 mμ and 265 mμ. It is known (45) that the hydroxyl function does not affect the sign of the Cotton effect but depending on its configuration does affect the position of the extrema. An axial hydroxyl group causes a bathochromic shift of 12 to 23 mμ for the first extrema.

Therefore, both the ORD data and the ultraviolet spectrum indicate that L.20 possess an axial hydroxyl group adjacent to a ketone.

The NMR spectrum of L.20 showed a peak at 9.17 τ (doublet, J = 5 cps) indicative of a secondary C-methyl group. There were nc olefinic protons. The high field position of the C-methyl peak is similar to that observed in lycopodine (9.16 τ) in which the methyl group lies somewhat in the shielding cone of the carbonyl group.

For these reasons it was suspected that L.20 was simply an α-hydroxylycopodine.

Chapman et.al. (40) have reported that α-(axial) ketols on reduction with calcium and liquid ammonia are converted to the parent ketones. This reaction was carried out on L.20, and lycopodine was obtained, thus establishing both the carbon skeleton and the position of the carbonyl group for alkaloid L.20.

The axial hydroxyl group could be located either on C-4 or C-6 of the lycopodine skeleton. Flabelliformine (XVII), an α-ketol, whose structure D. B. MacLean and M. Curcumelli-Rostomo (14) have recently determined, is the 4-α-hydroxy-lycopodine. Since these two compounds are not identical, the hydroxyl group must be on C-6, and structure XVIII represents L.20.

Further proof of the position of the hydroxyl group was obtained in the following many. α-Ketols in which the hydroxyl group is secondary are susceptible to aerial oxidation in the presence of base (47). Treatment of alkaloid L.20 under these conditions gave the enolic α-diketone LVI which had previously been prepared from lycopodine (48), thus establishing the location of both oxygen functions in alkaloid L.20.

The compound suspected to be the equatoral α-ketol LVIII epimeric at C-6 with alkaloid L.20 had been prepared (via the sequence shown on Chart VII, p. 28) in these laboratories in connection with structural studies on lycoclavine (LVII), an alkaloid of L. clavatum var. megastashyn (48). It therefore became of interest to interrelate the two compounds by epimerization of the hydroxyl group in L.20.

When L.20 was treated with sodium propoxide in n-propanol under anhydrous conditions and in the absence of oxygen, 6-β-hydroxylycopodine identical in all respects with that obtained from lycoclavine, was obtained. It showed infrared absorption (nujol) at 3250 cm^{-1} (-OH), 1723 cm^{-1} (ketone in six membered

ring). The ORD spectrum exhibited a positive Cotton effect [$[\alpha]^{MeOH}_{305}$ 272, $[\alpha]^{MeOH}_{255}$ - 6720], providing further proof for the configuration of the hydroxyl group.

Since at this time the stereochemistry at C-5 and C-6 in lycoclavine had not been established the two α-ketols available afforded an opportunity to settle this point by stereospecific reductions since presumably the four possible alcohols could be obtained (LIX→LXII as shown on Chart VIII, p. 40) one of which would be identical with the diol formed on hydrolysis of lycoclavine.

It is known (35) that lithium aluminum hydride reduction of lycopodine gives the axial epimer while lithium - ammonia reduction gives the equatorial epimer. Therefore, these two reduction methods were applied to L.20.

The lithium-methanol-ammonia (49) reduction of L.20 gave an unexpected product. Instead of a diol, epidihydrolycopodine (35) was obtained. This reduction first must proceed via the formation of the lycopodine as in the case of calcium-ammonia reduction mentioned previously, but in the presence of methanol and an excess of the reducing agent this is reduced to the alcohol.

The reduction of L.20 with lithium aluminum hydride gave the diol LXII which was identical with that obtained from the hydrolysis of lycoclavine. This result establishes the complete stereochemistry of lycoclavine, since, by analogy with the reduction of lycopodine, the hydroxyl group at C-5

produced by the reduction reaction must be axial. and it has been shown that the C-6 hydroxyl in L.20 is axial.

D.A. Law has shown that L.20 can be very readily synthesized by hydrolysis of 6- α -bromolycopodine with 5% $NaHCO_3$. Hydrolysis of the bromo compound, thus, takes place with retention of configuration. The mechanism by which the hydrolysis proceeds cannot be a simple S_n2 type displacement, since then the inverted, more stable ketol with the hydroxyl group in the equatorial position would be expected. Although α-haloketones usually hydrolyse very readily by this mechanism, in this case approach to the back side of C-6 is extremely hindered by the C-7, C-13 bridge LXIII. The observed retention of configuration would result if the reaction proceeded by attack of hydroxide at C-5 with the formation on an intermediate epoxy alcohol LXIV. Diaxial opening of the epoxide ring would lead to alkaloid L.20 LXV(R = H).

In order to investigate this possibility further we decided to investigate the action of methoxide on bromolycopodine, in the hope that in this case the intermediate epoxyether LXIV($R = CH_3$) might be isolated.

It is known, for example, that 1-methoxy-1-phenyl-2,2-dimethylethylene oxide LXVI (50) and 1-methoxy-1,2,2-triphenylethylene oxide LXVII (51) are obtained from the corresponding α-bromoketones when treated with sodium methoxide in methanol.

Therefore, 6-α-bromolycopodine hydrobromide was treated

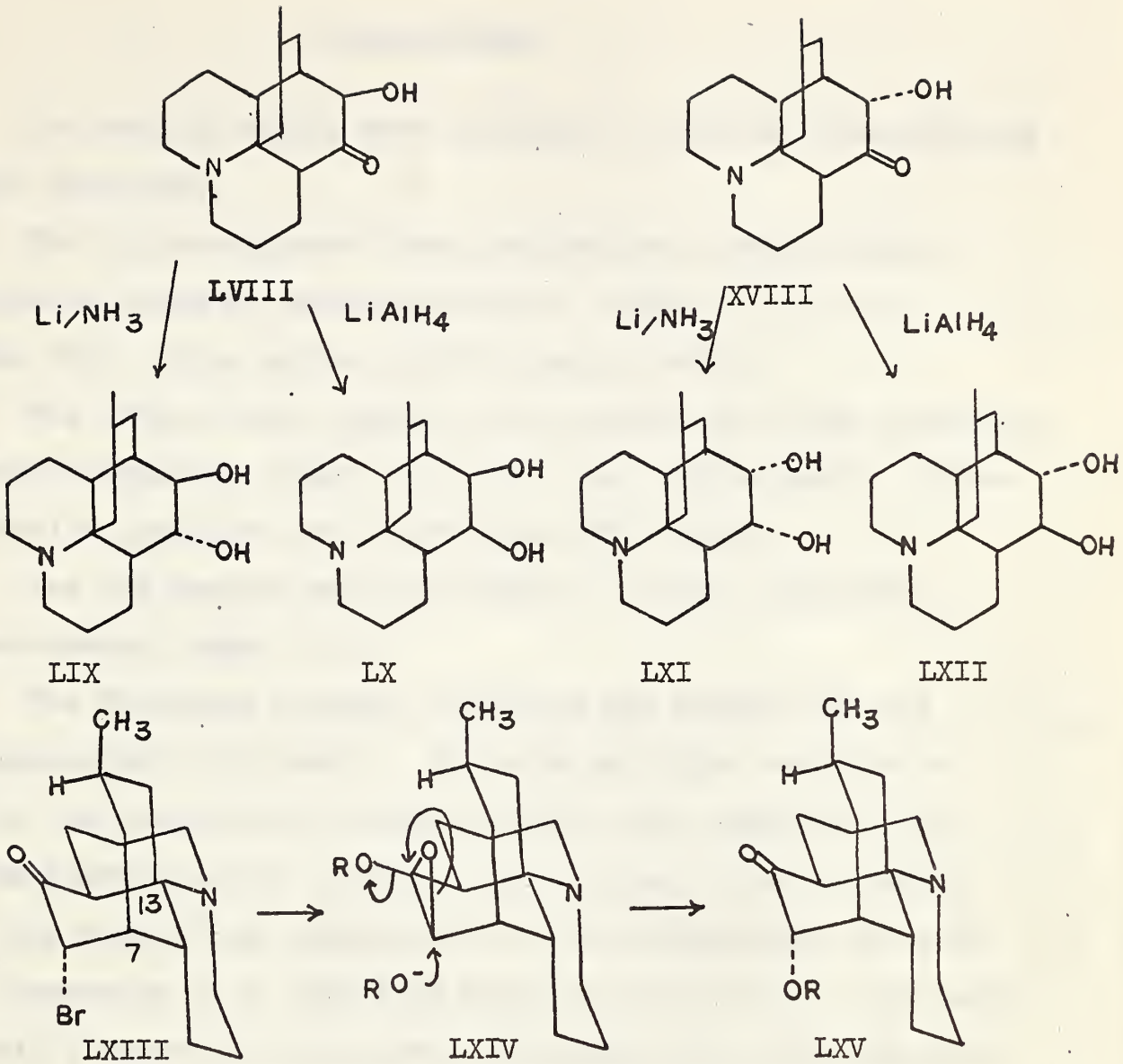
in anhydrous methanol with sodium methoxide. Instead of the epoxide an α-methoxy keto compound LXV($R = CH_3$), which analyzed for $C_{17}H_{27}O_2N$, was obtained. The infrared spectrum of the α-methoxy compound showed absorption at 1705 cm^{-1} (carbonyl of six membered ring ketone), 1173 cm^{-1} (medium, due to the methoxy group) and no hydroxyl absorption in 3600 - 3300 cm^{-1} region. The ultra-violet spectrum of the α-methoxy compound showed absorption at 305 $m\mu$ ($\log \epsilon$ 1.87), indicating the axial orientation of the methoxy group. The NMR spectrum showed a three proton signal at 6.78 τ due to the methoxy group. The ORD curve of the methoxy compound showed a positive Cotton effect ($[\alpha]_{330}^{MeOH}$ +1780 , $[\alpha]_{265}^{MeOH}$ - 4380).

The methoxy compound was characterized by formation of the methiodide. The infrared spectrum (nujol) showed absorption at 1712 cm^{-1} and a multiple peak centered at 1125 cm^{-1} due to the methoxy group. The ultra-violet spectrum showed a peak at 305 $m\mu$ ($\log \epsilon$ 1.84).

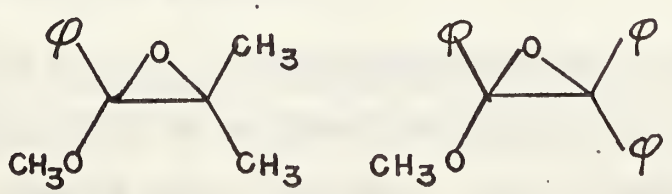
Attempts to isolate the intermediate epoxyether by treatment of the bromo compound with methoxide in non-hydroxylic solvents such as ether, dioxane, and chloroform were unsuccessful.

Finally it should be pointed out that the hydroxyl group in L.20 accurs at a "methyl carbon", that is, one derived from the methyl group of acetate, in the Conroy biogenetic scheme. Since this position is adjacent to the carbonyl, however, it is susceptible to oxidation and this finding may not be biogenetically significant.

CHART VIII



LXIII LXIV LXV



φ= phenyl group

LXVI LXVII

EXPERIMENTAL

The melting points were obtained on a Fisher-Johns Melting Point Apparatus.

The infrared spectra were recorded on a Perkin-Elmer Recording Infrared Spectrophotometer, Model P.E. 21 and Model 221G, using sodium chloride sealed cells.

The ultra-violet spectra were recorded on a Cary Recording Spectrophotometer, Model 14, using 1 cm. quartz cells. Unless otherwise specified the solvent was 95% ethanol.

The NMR spectra were recorded on a Varian Analytical Spectrometer, Model A 60.

The following standard procedure was adopted for the chromatography on alumina. The alumina column was made up with the least polar solvent in which the compounds to be chromatographed were soluble. Thirty grams of basic alumina per one gram of the substance to be chromatographed was used. The compounds to be separated were then dissolved in the least possible amount of the solvent with which the column was made up, and placed on the column. Only purified solvents were used for the eluting purposes.

PREPARATION OF 3,4,5,6,7,8-HEXAHYDROCARBOSTYRIL (XXVII)

A. PREPARATION OF 2-HYDROXYMETHYLENECYCLOHEXANONE (21)

Sodium metal (23 gms.), cut in approximately 1cm. cubes, dry ether (2 l.), redistilled cyclohexanone (1 mole, 98 gms.) and ethyl formate (1.5 moles, 110 gms.)

were added to a 5 l. three-necked flask equipped with a stirrer and a vent tube. The reaction was initiated by addition of ethyl alcohol (5 mls.), and the mixture was stirred for five hours. Then after addition of water (200 mls.), the mixture was shaken in a separatory funnel and the ether layer discarded. The aqueous extract was acidified with 6N hydrochloric acid, and the mixture was extracted twice with ether (300 mls.). The ether solution was washed with saturated sodium chloride solution (25 mls.) and then dried over anhydrous magnesium sulfate. After evaporation of the ether and distillation of the remaining yellow oil under reduced pressure 28.49 gms. (39% yield) of 2-hydroxy methylene cyclohexanone was obtained. Infrared spectrum ($CHCl_3$) showed peaks at 1595 cm^{-1} and 3480 cm^{-1}.

B. SYNTHESIS OF 3-AMIDO-5,6,7,8-TETRAHYDROCARBOSTYRIL (XXV) (20)

2-Hydroxymethylenecyclohexanone (9 gms.) and ethylcyanoacetate (8 gms.) were added to aqueous methanol (4 moles of water and 8 moles of methanol per mole of other reagents). The solution was saturated with ammonia and refluxed for four hours. Then the reaction was cooled and upon dilution with water, solid separated (8.11 gms., 60% yield) which upon recrystallization from acetic acid melted at 320°C. Infrared spectrum (nujol) showed absorption at 3100cm^{-1}, 3138 cm^{-1}, and 1672 cm^{-1}. The ultra-violet spectrum showed maxima at 240$m\mu$ ($\log \epsilon$ 3.88)

and 340mμ (log ε 4.0157).

C. PREPARATION OF 3-CARBOXY-5,6,7,8-TETRAHYDROCARBOSTYRIL (XXVI)

The amide (3.91 gms.) was dissolved in 80% H_2SO_4 (20 mls.) and then heated to reflux for one and a half hours. The hot solution was cooled and water added to it until no more precipitate formed. The 3-carboxy-5,6,7,8-tetrahydrocarbostyril (1.83 gms. 47% yield), m.p. 200°C, showed absorption in the infrared (nujol) at 2700 - 2500 cm^{-1} and 1695 cm^{-1}. Ultra-violet (95% EtOH) maxima were found at 237.5mμ (log ε 3.85) and 340mμ (log ε 3.99).

D. PREPARATION OF 5,6,7,8-TETRAHYDROCARBOSTYRIL (XXIV)

3-Carboxy-5,6,7,8-tetrahydrocarbostyril (0.18 gm.) was placed in a sublimation apparatus and heated under reduced pressure to 280°C. Instead of decarboxylation taking place, the acid sublimed, affording a useful purification procedure.

When the sublimed acid (0.15 gms.) was heated to the same temperature at atmospheric pressure decarboxylation took place smoothly, affording 5,6,7,8-tetrahydrocarbostyril (0.11gms). Infrared (nujol) showed absorption at 1660 cm^{-1}, 1630 cm^{-1}. Ultra-violet (95% EtOH) maxima occured at 230mμ (log ε 3.87) and 315mμ (log ε 3.84).

E. SYNTHESIS OF 3,4,5,6,7,8-HEXAHYDROCARBOSTYRIL (XXVII)(23)

A mixture of acrylonitrile (10.6 gms.) and cyclo-

hexanone (48 gms.) was added dropwise during 30 mins. to refluxing cyclohexanone (50 gms.) containing NaCN (1 cc. of a 50% aq. soln.). After refluxing for a further 15 mins., the solution was distilled to give cyclohexanone and two other fractions. The first of the two fractions was redistilled to give 2,2'-cyanoethylcyclohexanone (6.7 gms.) b.p. 121 /5mm.

2,2'-cyanoethylcyclohexanone (6.7 gms.) was refluxed for four hours with methanolic KOH (3 cc. of a 30% solution in t-butanol, 20 cc.). The semi-solid which separated after neutralization with dilute HCl and dilution with water was filtered, washed with ether and recrystallized from ethanol to give colourless needles of 3,4,5,6,7,8-hexahydrocarbostyril, (3.1 gms., m.p. 142°C.). Infrared (nujol) absorption occurred at 1669 cm^{-1} (s) with a shoulder at 1705 cm^{-1}. The ultra-violet (95% EtOH) maximum was at 252.5mμ (log ε 3.69).

(Found: C, 72.06; H, 8.95. $C_9H_{12}ON$ requires: C, 71.96; H, 8.66%.).

F. PREPARATION OF 3-CYANO-5,6,7,8-TETRAHYDROCARBOSTYRIL (XXXV) (27)

Cyanoacetamide (8.4 gms.) was dissolved in warm water (35 mls.) and freshly prepared 2-hydroxy-methylenecylohexanone (18 gms.) was added followed by enough alcohol to effect solution. Dimethylamine (2 mls.) was added and the solution was left overnight at room temperature. The crystals, which separated at

the end of this time were filtered (16.9 gms.) m.p. 218 - 80°C. The product was recrystallized from acetic acid, m.p. 218 - 80°C.

The product (1 gm.) was shaken with chloroform, in which it was partially soluble. The chloroform solution was filtered and the crystals remaining (0.71 gms.), which absorbed in the infrared (nujol) at 1685 cm^{-1} were identical to those of 3 amido-5,6,7,8-tetrahydrocarbostyril previously prepared.

The filtrate, after evaporation, left a residue, m.p. 247 - 248°C, (0.27 gms.) whose infrared spectrum (nujol) showed absorption at 2250 cm^{-1} (m) and 1647 cm^{-1}. The compound was 3-cyano-5,6,7,8-tetrahydrocarbostyril (melting point reported in literature, 249°C.).

PREPARATION OF THE YELLOW COMPOUND (XXXVII)

A. 2-Hydroxymethylenecyclohexanone (17 gms.) was added to a round bottom three-necked flask containing 1/2 mole of H_2O and one mole of methanol. Then the solution was saturated with NH_3 throughout the five hour period that the reaction mixture was refluxed. An hour before the reaction was stopped NH_4OH (50 mls.) was added to the reaction mixture. Towards the end of the reaction yellow crystals started to separate, which upon dilution of the reaction mixture with water and filtration yielded 5.5 gms. of yellow solid.

The compound after recrystallization from methanol melted at 155°C. Infrared (nujol) peaks were found at 1663 cm^{-1} and 1645 cm^{-1}. Maxima were found a 383mμ (log ε 4.46), 283mμ (log ε 3.42) and 253mμ (log ε 3.47) in the ultraviolet.

(Found: C, 72.33, 72.20; H, 8.14, 8.05; O, 14.13. $C_{14}H_{19}O_2N$ requires: C, 72.04; H, 8.21; O, 13.71%).

B. ATTEMPTED PREPARATION OF THE YELLOW COMPOUND BY THE METHOD OF MANSON ET AL. (30)

2-Hydroxymethylenecyclohexanone (12 gms.) was dissolved in benzene (500 mls.) and ammonia bubbled through for two hours. The reaction mixture was then left overnight at room temperature. Upon evaporation of the benzene, a light brown oil was obtained which partially crystallized. Crystallization from acetone-ether afforded colourless crystals (4 gms.), m.p. 115°C. Infrared spectrum (nujol): ν max 3340 cm^{-1} (m), 3273 cm^{-1} (w), 3173 cm^{-1} (s), 1655 cm^{-1} and 1615 cm^{-1}. Absorptrion in the ultraviolet occurred at 313mμ (log ε 4.27). The structure assigned to this product was that of 2-aminomethylenecyclohexanone, m.p. 110°C.

The ultraviolet spectrum of the mother liquors from the above operations did not show a peak at 383mμ, indicating that little, if any, of the "yellow compound" was formed.

C. SYNTHESIS OF THE YELLOW COMPOUND FROM 2-HYDROXYMETHYLENE-CYCLOHEXANONE AND 2-AMINOMETHYLENECYCLEXANONE.

2-hydroxymethylenecyclohexanone (3.5 gms.) and 2-amino-methylenecyclohexanone (3.5 gms.) were added to benzene (100 mls.) in a round bottom flask to which a water SEPARATOR with a condenser was attached. The reaction mixture was refluxed for 3 hours, during which time 1/2 ml. of water was collected. The solution was left at room temperature overnight, then the benzene was evaporated, leaving an orange-yellow semisolid (5.45 gms.) The ultraviolet spectrum showed intense absorption at 383 mμ and weaker absorption at 318 mμ and 280 mμ indicating the presence of the yellow compound, as well as that of 2-aminomethylenecyclohexanone and 2-hydroxymethylene-cyclohexanone.

The yellow compound, m.p. 155°C, was obtained by recrystallization of the orange-yellow semisolid. The infrared spectrum showed peaks at 1663 cm^{-1} and 1645 cm^{-1} and was identical with the spectrum of the yellow compound prepared previously.

D. HYDROGENATION OF THE YELLOW COMPOUND (XXXVII)

The yellow compound (0.30 gms.) and platinum oxide (0.05 gms.) were added to a flask containing ethanol (110 mls.) and hydrogenated under H_2 atmosphere (50 psi) for 16 hours. The platinum catalyst was then filtered off and the ethanol removed under reduced pressure. The

residue (0.28 gms.) a light coloured oil, crystallized from ethyl acetate as colourless crystals, m.p. 85°C. The infrared spectrum (nujol) showed absorption at 3380 cm^{-1}, 3250 cm^{-1}, 3100 cm^{-1} (w) and 1600 cm^{-1}. Ultraviolet maximum occurred at 328mμ (log ε 4.35). The product, a monosubstituted enamine was assigned structure XL.

(Found: C, 70.94, 71.09; H, 9.82, 10.10; O, 13.39. $C_{14}H_{23}O_2N$ requires: C, 70.88; H, 9.71; O, 13.5%)

E. $NaBH_4$ REDUCTION OF YELLOW COMPOUND (XXXVII)

The yellow compound (1.84 gms.) was dissolved in methanol (100 mls.) and a solution of $NaBH_4$ (500 mgs. in 15 mls. of H_2O and 1 ml. of 5% NaOH) was added. The reaction mixture was refluxed for four hours. The methanol was then partially evaporated, and the residue basified with dilute NH_4OH and extracted with chloroform. Upon drying the chloroform with anhydrous megnesium sulfate and evaporating, a yellow oil was obtained (1.74 gms, 92% yield). Infrared spectrum (CCl_4) showed a weak band at 3622 cm^{-1}, a broad band with a peak at 3300 cm^{-1} and no carboxyl absorption. The compound showed no maximal absorption above 215mμ in the ultraviolet.

(Found: C, 69.76, 69.64; H, 10.84, 10.94. $C_{14}H_{27}O_2N$ requires: C, 69.65; H, 11.23%).

F. HYDROLYSIS OF THE YELLOW COMPOUND (XXXVII)

Yellow compound (.92 gms.) was hydrolyzed with 5% NaOH (53 mls.) The reaction mixture was refluxed until the yellow colour imparted by the compound to the solution disappeared (72 hours). Throughout this period NH_3 could be detected escaping. At the end of this time, the reaction mixture was further diluted with water and extracted with chloroform, which was then dried over anhydrous magnesium sulfate, and evaporated to give a light yellow oil (.42 gms.). The infrared spectrum ($CHCl_3$) showed absorption at 1710 cm^{-1}, and was superimposable upon that obtained of cyclohexanone.

The light oil was added to a hot solution of 2,4-dinitrophenylhydrazine (10 mls. of EtOH, .942 gms. of 2,4-dinitrophenylhydrazine and a few drops of HCl). The solution was then left to stand until an orange 2,4-dinitrophenylhydrazone separated. After recrystallization from ethanol, the 2,4-dinitrophenylhydrazone of the oil obtained above was identical with the 2,4-dinitrophenylhydrazone prepared similarly from cyclohexanone in, m.p. 161 C., mixed melting point and infrared spectrum.

THE MINOR ALKALOIDS OF LYCOPODIUM CLAVATUM L.

The material used for this examination consisted of the basic material remaining after the removal of most of the lycopodine (by crystallization of the acetone-insoluble perchlorate) from the crude alkolids of L. clavatum Linn. The extraction of the crude alkaloids was carried out by D.A. Law using the method of Manske and Marion. A total of 27 grams of crude minor alkaloids was chromatographed as shown below:

Fraction	Eluant	Wt. of Fraction
I	benzene (1 L.)	4.08 gms.
II	benzene - methanol (400:1)(1L.)	2.73 gms.
III	benzene - methanol (200:1)(1L.)	1.20 gms.
IV	benzene - methanol (100:1)(1L.)	0.89 gms.
V	benzene - methanol (50:1)(1L.)	0.61 gms.
VI	benzene - methanol (25:1)(1L.)	8.21 gms.
VII	benzene - methanol (25:2)(1L.)	3.53 gms.
VIII	benzene - methanol (5:1)(1L.)	0.17 gms.
IX	benzene - methanol (5:2)(1L.)	0.31 gms.
X	methanol	0.30 gms.

Further separation of alkaloids was achieved by fractional crystallization.

Fraction I. Crystallization from acetone yielded lycopodine (1.68 gms.), m.p. 114 - 5°C, identical with an authentic sample (m.p., m.m.p. and infrared spectra).

Fraction II. Crystallization from acetone also yielded lycopodine (0.44 gms.), m.p. 116°C, identical in all respects with an authentic sample (m.p., m.m.p. and superimposable infrared spectra).

Fraction III, IV, V. Crystallization of the three fractions mentioned from ether yielded lycodine, m.p. 117 - 18°C, 0.29 gms., 0.12 gms. and 0.10 gms. respectively. The infrared spectra showed bands at 3265 cm^{-1} (N-H) and 1575 cm^{-1} (medium) and was identical to that of an authentic sample kindly provided by G.G. Iverach. The ultraviolet showed a maximum at 268 mμ with a shoulder at 276mμ. The lycodine was further characterized by preparation of N-methyllycodine.

PREPARATION OF N-METHYLLYCODINE

Lycodine (200 mgs.) was dissolved in 98% formic acid (1.64 mls.) and 40% formaldehyde (1.64 mls.) and the solution was refluxed for 4 hours. At the end of this time the solution was evaporated to dryness and the residue (190 mgs.) was chromatographed on alumina giving white crystalline material (with ether, 180 mgs.), m.p. 91-91.5°C. The infrared spectrum (nujol) lacked the N-H band of lycodine in the 3260 cm^{-1} region but showed absorption at 1573 cm^{-1} (m-s). The N-methyllycodine thus prepared was identical with N-methyllycodine obtained from natural sources as indicated by melting point, mixed melting point and superimposable infrared spectra.

Fraction VI

a) Crystallization from acetone yielded white crystalline material (0.74 gms.), m.p. 208°C., which absorbed in the infrared at 3250 cm^{-1} (shallow, broad band) and 1707 cm^{-1}. The 208° compound analyzed for $C_{32}H_{52}N_2O_3$.

(Found: C, 74.75, 74.97; H, 10.06, 10.12; N, 5.56; O, 9.59

$C_{32}H_{52}N_2O_3$ requires: C, 74.92, H, 10.22, N, 5.46; O, 9.40%)

Chromatography over alumina indicated that the 208° compound is a single component. Proper chromatography on Whatman paper No. 4 with aqueous butanol gave a single spot (developed with Dragendorf's reagent).

SARETT OXIDATION OF THE 208°C ALKALOID

The 208° alkaloid (o.13 gms.) was dissolved in pyridine (2.5 mls) and was added to pyridine - CrO_3 complex (pyridine, 2.5 mls.; CrO_3, .13 gms.). The reaction mixture was shaken for two hours and then left overnight at room temperature. The reaction mixture was then diluted with water, further basified with NH_4OH and extracted with chloroform. The chloroform solution was dried and the solvent was removed under reduced pressure giving a partially crystalline product (.071 gms.). The product was repeatedly crystallized from acetone, m.p. 165°C, and showed absorption in the infrared (nujol) at 1710 cm^{-1} and 1695 cm^{-1} and did not show any absorption in the hydroxyl region. The product analyzed for $C_{32}H_{50}NO_3$.

(Found: C, 75.51; H, 9.92. $C_{32}H_{50}NO_3$ requires: C, 75.24; H, 9.87%).

Chromatogaphy over alumina separated the 165° product into lycopodine (eluted with benzene) and flabelliformine (eluted with benzene-ether, 1:1).

Combination of equimolar quantities of dihydrolycopodine and flabelliformine in acetone (experiment performed by D.A. Law) regenerated the 208° complex, identical in all respects to that isolated from the plant.

b) From fraction VI, by crystallization from acetone, a second substance (0.09 gms.), was obtained which melted at 214°C. The infrared spectrum was similar to that of lycopodine but showed a band 3430 cm^{-1} (m). The compound was identical with flabelliformine as indicated by m.p., mixed m.p. and superimposable infrared spectra.

c) Fraction VI also yielded a small quantity of a substance (0.02 gms.), m.p. 268 - 72°C, by further concentration of the acetone mother liquors. The compound was identified as de-N-methyl α-obscurine as will be discussed later.

The mother liquors of Fraction VI, after the removal of the above crystalline substances, will be referred to as Fraction VI A.

Fraction VII: Crystallization from acetone yielded a substance (0.17 gms.), m.p. 240°C. Infrared absorption (nujol) was found at 3300 cm^{-1} and 1710 cm^{-1}. The compound was identical with clavolonine as indicated by m.p., mixed m.p. and superimposable infrared spectra.

Fraction VI A (5.75 gms.) was chromatographed over alumina of activity IV, as follows:

Fraction	Eluant	Wt. of fraction
I B	benzene (500 mls.)	1.12 gms.
II B	benzene - ether (100:1)(500 mls.)	0.78 gms.
III B	benzene - ether (25:1)(500 mls.)	0.42 gms.
IV B	benzene - ether (5:1) (500 mls.)	0.43 gms.
V B	benzene - ether (2.5:1)(500 mls.)	0.62 gms.
VI B	ether (500 mls.)	0.53 gms.
VII B	ether - chloroform (100:1)(500 mls.)	0.29 gms.
VIII B	ether - chloroform (25:1)(500 mls.)	0.093 gms.
IX B	ether - chloroform (5:1)(500 mls.)	0.012 gms.
X B	ether - chloroform (2.5:1)(500 mls.)	0.081 gms.
XI B	chloroform (500 mls.)	0.42 gms.
XII B	chloroform - methanol (100:1)(500 mls.)	0.86 gms.
XIII B	chloroform - methanol (25:1)(500 mls.)	0.13 gms.
XIV B	chloroform - methanol (5:1)(500 mls.)	0.048 gms.
XV B	chloroform - methanol (2.5:1)(500 mls.)	0.015 gms.
XVI B	methanol	0.032 gms.

Fraction V B. Crystallization from acetone yielded a substance (0.18 gms.), m.p. 180°C. The infrared spectrum showed absorption at 3325 cm^{-1}, 1712 cm^{-1} and 1696 cm^{-1}. The compound analyzed as $C_{16}H_{25}O_2N$.

Found: C, 73.72, 73.69; H, 9,70, 9.54; H, 11.81. $C_{16}H_{25}O_2N$ requires: C, 73.11; H, 9.16; O, 12.16%.

The 180°C substance was carefully chromatographed over alumina. Elution with ether separated substance into lycodoline (eluted first) and flabelliformine.

Paper chromatography of the compound was carried out on Whatman paper No. 4 using water saturated n-butanol as the mobile phase and gave spots having Rf values of 0.52 and 0.76 which were identical with the Rf values found for lycodoline and flabelliformine respectively. Therefore, the 180° substance is a complex of flabelliformine and lycodoline (1:1)

Fraction VI B Crystallization from acetone yielded a compound (0.020 gms.), m.p. 150-52°C. The infrared spectrum (nujol) did not show any absroption in the carbonyl region but a band was found at 3350 cm^{-1} (s). The compound was identical with dihydrolycopodine as indicated by m.p., mixed m.p. and superimposable infrared spectra.

Fraction VII B Crystallization from acetone yielded a substance (10 mgs.), m.p. 268 - 276°C, whose infrared spectrum (nujol) showed absorption at 1700 cm^{-1} and 1680 cm^{-1}. The compound was identical with α-obscurine as indicated by m.p., mixed m.p. and superimposable infrared spectra.

Fraction XII B. Crystallization from ethyl acetate yielded crystalline material (150 gms.), m.p. 268-72°C. The infrared absorption (nujol) was found at 3250 cm^{-1} (NH), 1700 cm^{-1} (shoulder), 1678 cm^{-1} and 1644 cm^{-1}. The ultraviolet spectrum showed a maximum at 255 $m\mu$ ($\log \epsilon$ 3.8). The substance was

identical with de-N-methyl-α-obscurine prepared from α-obscurine as indicated by m.p., mixed m.p. and superimposable infrared spectra.

Methylation of de-N-methyl obscurine (XXIV)

De-N-methyl-α-obscurine (30 mgs.) was refluxed for four hours with 98 formic acid (0.25 mls.) and 40% formaldehyde (0.25 mls.). Dilution with water, basification (NH_4OH) and extraction with chloroform, yielded α-obscurine (29 mgs.), identical in m.p., mixed m.p. and infrared spectrum with an authentic sample.

Fractions VIII to X, as well as the remaining fractions, could not be induced to crystallize, even (in most cases) after further chromatography. Attempts to obtain crystalline salts with perchloric acid and hydrobromic acid were also unsuccessful. The remaining oily material was combined and reserved.

A. PREPARATION OF DIKETONE FROM ANNOFOLINE (XLII)

a. CHROMIUM TRIOXIDE OXIDATION OF FAWCETTIINE (L.)

Fawcettiine (.94 gms.) was dissolved in pyridine (10 mls.) and added to chromium trioxide pyridine complex (1.00 gms. of CrO_3, 10 mls. of pyridine) at 0°C. The reaction mixture was stirred for one and a half hours and then left to stand overnight at room temperature. Then the reaction mixture was further basified with dilute NH_4OH and extracted with chloroform (4 x 100 mls.).

The chloroform solution was dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. A light coloured oil was obtained (.74 gms.), which crystallized from acetone, m.p. 135°C. The infrared (nujol) showed absorption at 1738 cm^{-1}, 1704 cm^{-1} and 1228 cm^{-1}.

b. DEACETYLATION OF OXAFAWCETTIINE (LII)

Oxofawcettiine (.300 gms.) was dissolved in MeOH (25 mls.) to which 5N NaOH (50% aqueous methanol)(30 mls.) was added. The reaction mixture was left at room temperature for 15 hours. The reaction mixture was then diluted with water and extracted with chloroform (4 x 100 mls.). The chloroform solution was dried over anhydrous magnesium sulfate, and evaporated under reduced pressure at the steam bath giving a light oil (.291 gms.) which crystallized from acetone as colorless plates melting at 138-140°C. The infrared spectrum in nujol showed absorption at 3460 cm^{-1} (-OH), 1702 cm^{-1} (six-membered ketone). The spectrum was identical to that of

authentic spectrum of annofoline kindly furnished by Dr. F.A.L. Anet.

c. OXIDATION OF ANNOFOLINE (XLII) CrO_3/ACOH AND CrO_3 (450mgs) ADDED

Annofoline (450 mgs.) was dissolved in 95% AcOH (10 mls.). The reaction mixture was stirred for one hour and then it was diluted with water, basified with dilute NH_4OH and extracted with chloroform. The chloroform solution was then dried over anhydrous magnesium sulfate and evaporated under reduced pressure giving an oil (364 mgs., 81.5% yield). Infrared spectrum absorbed at 1750 cm^{-1}, 1719 cm^{-1} and 1420 cm^{-1}.

The oily product was chromato graphed over alumina. Elution with dichloromethane gave the diketone (130 mgs, 29%). The infrared spectrum (CCl_4) of the diketone showed peaks at 1700 cm^{-1} and 1418 cm^{-1}.

d. OXIDATION OF ANNOFOLINE WITH CHROMIUM TRIOXIDE PYRIDINE

Annofoline (.28 gms.) was dissolved in pyridine (10 mls.) and was added to CrO_3-pyridine complex (CrO_3, 1.5 gms.; pyridine (10 mls.) at 0°C. The reaction mixture was allowed to reach room temperature and to stand with stirring for one hour.

Then it was diluted with water and basified with 5% NaOH solution. The aqueous solution was extracted with chloroform, which was then dried over anhydrous magnesium sulfate and evaporated giving a colourless oil (.20 gms., 70% yield).

The oil was then chromatographed on alumina.

The diketone was eluted from the column with dichloromethane. Infrared spectrum (CCl_4) of the diketone showed peaks at 1700 cm^{-1} and 1418 cm^{-1} (sharp). The diketone obtained thus was identical with that obtained from oxidation with CrO_3 in AcOH. The analytical sample was prepared by molecular distillation of the chromatographically pure material.

Found: C, 72.95, 73.11; H, 8.92, 8.77. Required for $C_{16}H_{23}O_2N$: C, 73.57; H, 8,81%.

e. PREPARATION OF METHIODIDE OF ANNOFOLINE DIKETONE (XLVIII)

Annofoline diketone (20 mgs) was dissolved in acetone to which methyl iodide(1/2 mls.) was added. The reaction mixture was refluxed for 20 minutes. On cooling white crystals separated, which after recrystallizedfrom MeOH, melted at 303°C.

Found: C, 50,80; H, 6.96. $C_{17}H_{26}O_2NI$ Requires: C, 50.62; H, 6.4%,

B. PREPARATION OF DIKETONE FROM CLAVOLONINE

a. DEACETYLATION OF FAWCETTIINE (L)

Fawcettiine (490 mgs.) was dissolved in methanol (50 mls.) to which 50% methanolic 5N NaOH (35 mls.) was added. The solution was left to stand for 15 hours at room temperature. Then the reaction mixture was diluted with water and extracted with chloroform. The chloroform was dried and removed under reduced pressure.

White powdery material separated (400 mgs., 95% yield)

which was crystallized from acetone, m.p. 203° C. (The literature (52) gives m.p. 203 - 204°C.) The infrared spectrum showed absorptiom at 3450 cm^{-1} (strong, sharp) and 3350 cm^{-1} (broad) and nc carbonyl absorption.

b. PREPARATION OF CLAVOLONINE (XLI)

Deacetylfawcettiine (0.39 gms.) was dissolved in pyridine (6 mls.) and added to CrO_3 -pyridine complex (CrO_3, .6 gms.; pyridine, 6 mls.) at 0° C. The solution was agitated for 1 1/2 hrs. at room temperature. Then the reaction mixture was diluted with water, further basified with dilute NH_4OH and extracted with chloroform. The chloroform solution was dried over anhydrous magnesium sulfate and evaporated under reduced pressure. A light yellow oil was obtained (.23 gms. 67% yield) which crystallized readily on standing. Recrystallization from acetone afforded pure c lavolonine, m.p. 240°C.

The infrared spectrum (nujol) showed absorption at 3200 cm^{-1} (broad band), 1695 cm^{-1} and was identical to that of clavolonine.

c. OXIDATION OF CLAVOLONINE (XLI) TO THE DIKETONE

Clavolonine (300 mgs.) was dissolved in pyridine (10 mls.) and added to CrO_3-pyridine complex (CrO_3, 1.5 gms; pyridine, 10 mls.) at 0°C. The solution was left to stand at room temperature for one hour. The reaction mixture was diluted with water, basified with 5% NaOH and extracted with chloroform. The chloroform extract was dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The

remaining pyridine was removed in vacuo, yielding a light brown oil (198 mgs.). Infrared spectrum ($CHCl_3$) of the oil showed bands at 3005 cm^{-1} (sharp), 3400 cm^{-1} (broad), 1750 cm^{-1} and 1715 cm^{-1}.

Chromatography over alumina and elution with CH_2Cl_2 afforded the diketone (128 mgs.). The infrared spectrum (CCl_4) was identical to that of the diketone prepared by oxidation of annofoline, and was further characterized by conversion to the methiodide.

d. METHIODIDE OF CLAVOLONINE DIKETONE

The diketone (25 mgs.) was dissolved in acetone (3 mls.) to which methyl iodide (1/2 mls.) was added. The solution was refluxed 20 mins. On cooling white crystals separated. The crystalline methiodide was recrystallized from methanol, m.p. 297 - 298°C.

Infrared in nujol showed absorption at 1733 cm^{-1} and 1712 cm^{-1}.

Mixed melting point of annofoline and clavolonine diketone methiodides did not show any depression and their infrared spectra were superimposable.

e. $LiAlH_4$ REDUCTION OF THE DIKETONE OF ANNOFOLINE (XLVIII)

Annofoline diketone (39 mgs.) was dissolved in freshly distilled tetrahydrofuran, (10 mls.) and $LiAlH_4$ (50 mgs.) was added showly. The condenser was equipped with a drying tube and the reaction mixture was refluxed for four hours. Excess $LiAlH_4$ was destroyed by addtion of ethylacetate.

The solution was diluted with water and continuously extracted with ether. The ethereal extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give crystalline deacetyl-fawcettiine (22 mgs. 58% yield) m.p. 204°C.

The mixed melting point of the above reaction product with an authentic sample of deacetylfawecttiine did not show any depression and the infrared spectrum (nujol) was also completly superimposable on that of deacetylfawcettiine.

C. PREPARATION OF EPICLACOLONINE (LV)

a. HYDROLYSIS OF α-LOFOLINE (LIII)

α-Lofoline (250 mgs.) was dissolved in methanol (20 mls.) to which 5% NaOH (10 mls.) was added. The reaction mixture was left at room temperature for 24 hours. The solution was then diluted with water and extracted with chloroform. The chloroform solution was dried over anhydrous magnesium sulfate and evaporated under reduced pressure giving a colourless oil (210 mgs., 95% yield) which failed to crystillize.

The infrared spectrum ($CHCl_3$) showed absorption at 3615 cm^{-1} (s), 3425 cm^{-1} (m), shallow, broad band) and no carbonyl absorption.

b. PREPARATION OF EPICLAVOLONINE

Deacetyllofoline (180 mgs.) was dissolved in pyridine (2 mls.) and CrO_3-pyridine complex (140 mgs. of CrO_3; 2mls of pyridine) added at 0°C. The reaction mixture was left at room temperature for one hour. The solution then was diluted with water, further basified with dilute NH_4OH and

extracted with chloroform. The chloroform solution was dried over magnesium sulfate and evaporated under reduced pressure. Crystallization from acetone afforded colourless crystals (126 mg.) m.p. 206 - 207°C. Infrared spectrum (nujol) $\nu_{max.}$ 3195 cm^{-1} (s), 1707 cm^{-1} and 1420 cm^{-1} (w). A CCl_4 solution showed bands at 3640 cm^{-1} (s), 3325 cm^{-1} (broad, medium), 1708 cm^{-1}, 1420 cm^{-1} (s) and 1022 cm^{-1} (very strong).

The analytical sample was prepared by crystallization from acetone followed by sublimation.

Found: C, 73.02, 72.60; H, 9.47, 9.48

$C_{16}H_{25}O_2N$ requires: C, 73.01; N, 9,50%.

EXTRACTION AND SEPARATION OF THE ALKALOIDS FROM L. LUCIDULUM MICHX.

Dried, finely ground plant material (24 lbs.) was Soxhlet extracted with methanol and the extracted concentrated to a thick, dark residue. The residue was digested for 24 hrs. with dilute hydrochloric acid. Then the acidic solution was filtered, and the acidic and neutral components were removed from the filtrate by ether extraction. The acidic solution was then basified with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried and evaporated, yielding the crude alkaloidal material (120 gms., 0.9%). Further purification of the alkaloidal material was achieved by a second acid base extraction.

The alkaloidal material (110 gms.) was separated into weak and strong bases by adjusting the pH of an aqueous acid solution to 8, then extracting with chloroform to recover the weak bases. The pH was then raised to about 10 with concentrated ammonium hydroxide and the strongly basic material extracted with chloroform.

The weakly basic alkaloids (87.75 gms.) showed absorption in the infrared ($CHCl_3$) at 1700 cm^{-1} (weak) 1628 cm^{-1}(strong). In the ultraviolet, a low intensity peak was observed at 298 $m\mu$.

The strongly basic alkaloids (19.14 gms.) absorbed in the infrared ($CHCl_3$) at 1720 cm^{-1} (strong) and 1650-1620 cm^{-1} (weak shoulder). Weak ultraviolet absorption was found in the 250-260 mμ region. The weakly basic material was further separated by countercurrent distribution. The weak bases (37 gms.) were distributed between aqueous phosphate buffer (pH 6) and chloroform (moving phase) in 10 separatory funnels. Twenty fractions were obtained which yielded unknown alkaloid (33 gms, first three fractions, the weak bases) and lycopodine (4 gms., the remaining 17 fractions, the strong bases).

Unknown alkaloid showed absorption in the infrared ($CHCl_3$) at 1620 cm^{-1} and in CCl_4 at 1650 cm^{-1}. All attempts to crystallize material or to prepare crystalline derivatives (hydrobromide, hydrochloride, perchlorate, methiodide, picrate and picrolonate) were unsuccessful. Chromatography over alumina failed to affect further purification. The analytical sample was prepared by molecular distillation (160/0.5mm.).

Found: C, 73.34, 72.86; H, 10.39, 10.39; N, 7.98.
Calculated for $C_{26}H_{38}O_2N_2$: C, 74.17; H, 10.38; N, 7.20%.

The strong bases were dissolved in MeOH from which white crystalline material (300mgs.) separated. On recrystallization from methanol colorless crystals, m.p. 250°C were obtained. Infrared spectrum (nujol): $\nu_{max.}$ 3100 cm^{-1}, 2800-2500 cm^{-1}, and 1722 cm^{-1}. Ultraviolet

spectrum: λ max. 296mμ (log ε 1.85). The infrared (nujol) was identical to that of alkaloid L. 20 (authentic sample furnished by Dr. L. Marion) and a mixed melting point showed no depression.

The optical rotatory dispersion curve (Figure 4) showed a positive Cotton effect with extrema at 322.5 ($[\alpha]^{MeOH}_{322.5}$ 1500) and 281mμ ($[\alpha]^{MeOH}_{281}$ -3400).

The remainig strong bases were chromatographed over alumina. The ether eluted fractions yielded crystalline materials which were identified as lycopodine (0.75 gms.) and lycodoline (0.85 gms.). This is the first time that the separation of lycodoline from L. lucidulum has been reported. The remaining fractions failed to crystallize.

THE STRUCTURE OF ALKALOID L. 20

REDUCTION OF L. 20 WITH CALCIUM AND LIQUID AMMONIA (46)

L. 20 (97mgs.) was dissolved in tetrahydrofuran (7mls.) and added to a blue solution of calcium (100 mgs.) in liquid ammonia (150 mls.). The reaction mixture was stirred for 15 mins. The blue colour persisted throughout the reaction time. Excess calcium was removed by addition of bromobenzene and then water (5 mls.). After most of the ammonia had evaporated water was added and the aqueous solution was extracted with chloroform. The chloroform solution was dried over anhydrous magnesium sulfate and evaporated giving light brown oil (71.9 mgs.). The infrared spectrum ($CHCl_3$) showed absorption at 3350 cm^{-1} and 1698 cm^{-1}.

The oil was chromatographed over alumina giving lycopodine (43 mgs, dichloromethane), epidihydrolycopodine (20 mgs, chloroform) and the diketone XLVI (3mgs, methanol:chloroform 1:20).

EPIMERIZATION OF L. 20 TO THE LYCOCLAVINE KETOL (LVIII)

To anhydrous n-propanol, through which N_2 was bubbled for several minutes and which was kept under an N_2 atmosphere at all times, sodium propoxide (80 mgs.) and then alkaloid L. 20 (45 mgs.) were added. The solution was shaken at room temperature for 43 hours.

At the end of this time, the reaction mixture was diluted with water, 5% $NaHCO_3$ added and the resulting solution extracted with chloroform, which was dried and evaporated to give white crystalline material. After crystallization from acetone, the crystalline material melted at 120°C and the infrared spectrum (nujol) showed absorption at 3250 cm^{-1}, and 1725 cm^{-1}. The ultraviolet spectrum showed maximum at 280 mμ (log ε 1.90); Optical rotatory dispersion spectrum: ($[\alpha]^{MeOH}_{305}$ 2720, $[\alpha]^{MeOH}_{255}$ -6720). The compound was identical in all respects to the lycoclavine ketol (LVIII). The infrared spectra were superimposable and mixed m.p., did not show any depression.

LITHIUM-METHANOL-AMMONIA REDUCTION OF L. 20 (49)

L. 20 (250 mgs) was dissolved in methanol (5 mls) and the solution was added to a flask containing liquid ammonia (150 mls). Over a period of 15 minutes Li metal (500 mgs) was added to the solution. Throughout the reaction the blue colour in the reaction mixture persisted.

Then ammonium chloride (2.5 gms) was added and the ammonia was evaporated. The residue was then diluted with water and extracted with chloroform which was dried and evaporated giving light brown oil (180 mgs). The infrared spectrum ($CHCl_3$) showed absorption at: 3350 cm^{-1} and no carbonyl absorption.

Chromatography on alumina afforded crystalline epidihydrolycopodine, m.p. 137-38°C.

An acetone solution of the compound was treated with gaseous hydrogen chloride. The infrared spectrum of the epidihydrolycopodine hydrochloride thus obtained was superimposable on that of an authentic sample.

LITHIUM ALUMINUM HYDRIDE REDUCTION OF L. 20

L. 20 (25 mgs) was suspended in ether to which $LiAlH_4$ (50 mgs) was added. The slurry was refluxed for 48 hours. After addition of dilute ammonium hydroxide, the reaction mixture was continuously extracted with ether for 72 hours.

The ether was dried and evaporated giving light coloured oil (22 mgs).

The perchlorate, m.p.290-95°C, of the base was prepared in acetone. The infrared spectrum of the perchlorate (nujol) showed peaks at 3350 cm^{-1} and 2500 cm^{-1} (N^+) but no carbonyl absorption. This infrared spectrum was identical with that of the perchlorate of the diol obtained on hydrolysis of lycoclavine.

PREPARATION OF 6-α-BROMOLYCOPODINE HYDROBROMIDE (LXIII)

Lycopodine hydrobromide (3.18 gms) was dissolved in chloroform (60 mls) containing .1 ml of chloroform saturated with HBr, and bromine (1 gm in 15 mls of chloroform) was added dropwise. The reaction mixture was stirred at room temperature for 24 hours. The chloroform was evaporated under reduced pressure and the solid remaining was washed with acetone and filtered. In this way 3.38 gms. of bromolycopodine hydrobromide was obtained. The infrared spectrum (nujol) showed absorption at 2500 cm^{-1} ($N\overset{+}{H}$) and 1718 cm^{-1} and was identical with that of an authentic sample of 6-α-bromolycopodine hydrobromide.

PREPARATION OF METHOXY COMPOUND LXV ($R=CH_3$) (50)

6-α-Bromolycopodine hydrobromide (940 mgs.) was added to anhydrous methanol (25 mls) containing sodium methoxide (300 mgs.). After 15 minutes the reaction mixture was diluted with a large volume of chloroform (150 mls), which was washed with water (25 mls.) to remove inorganic material. After drying and evaporating the chloroform, a brown oil was obtained (475 mgs). Crystallization from ether afforded colorless crystals, m.p. 120-21°C, which showed absorption in the infrared (nujol) at 1705 cm^{-1} and 1173 cm^{-1}. The ultraviolet spectrum showed a maximum at 304mμ (log ε 1.87); ORD: $([\alpha]^{MeOH}_{330}$ 1780, $[\alpha]^{MeOH}_{265}$ -4380).

Found: C,73.24; H,9.77. $C_{17}H_{27}O_2N$ requires: C,73.77; H, 9.75%.

The methoxy compound obtained above (450 mgs.) was dissolved in methanol to which methyl iodide (2.5 mls) was added. The reaction mixture was refluxed for twenty minutes. At the end of this time crystals separated. The methiodide obtained was recrystallized from methanol, m.p. 300° C. The infrared spectrum showed absorption at 1712 cm^{-1} and 1125 cm^{-1}. The ultraviolet maximum was found at 305 mμ (log ε 1. 84).

Found: C, 50.99, 50.85; H, 6.81. 6.93. Calculated for $C_{12}H_{30}O_2NI$: C,51.52; H, 7.14%.

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ULTRAVIOLET SPECTRA

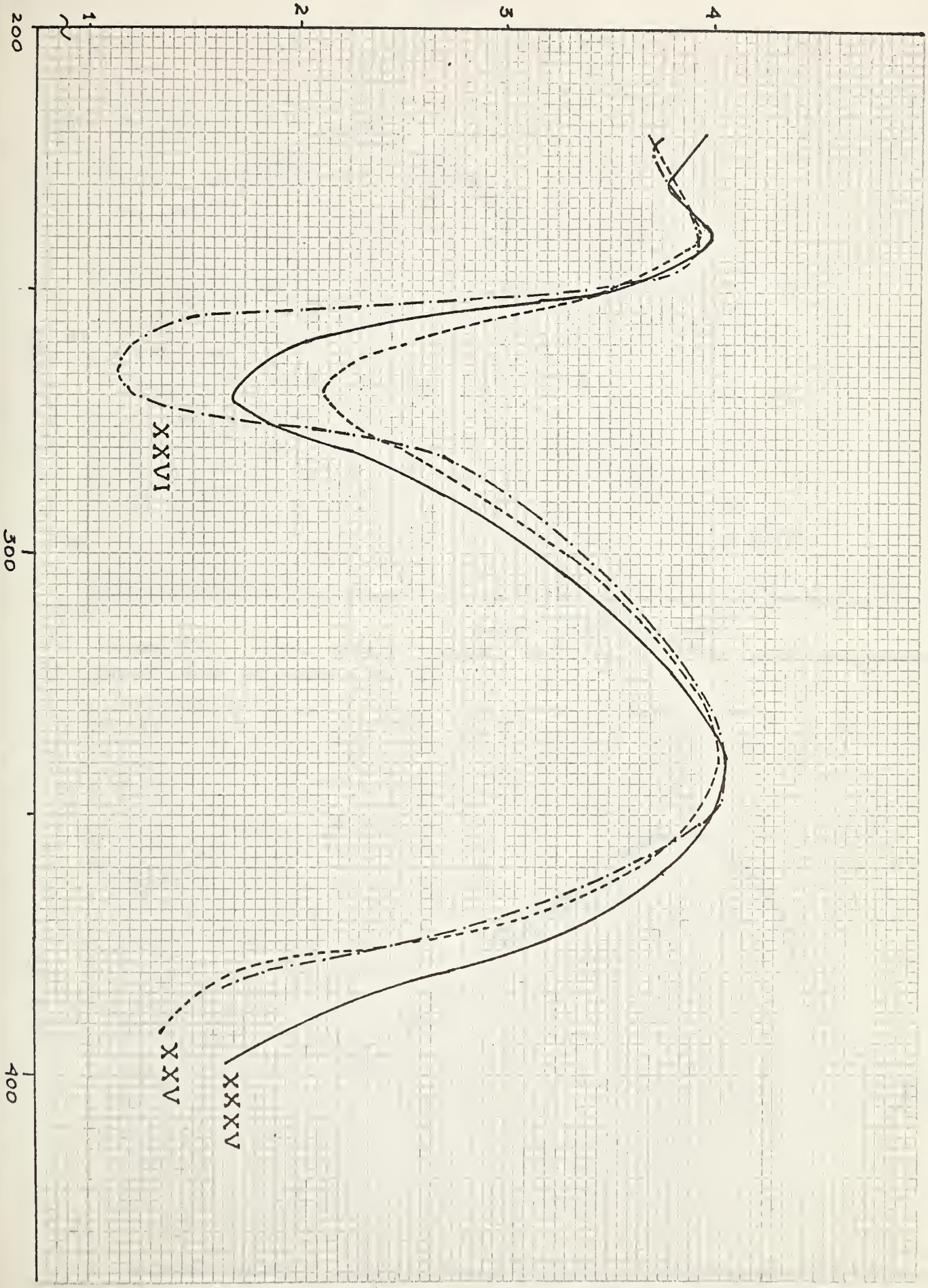


Figure I. Ultraviolet spectra in ethanol of 3-cyano-5,6,7,8-tetrahydrocarbostyril (XXXV), 3-amido-5,6,7,8-tetrahydrocarbostyril (XXV) and 3-carboxy-5,6,7,8-tetrahydrocarbostyril (XXVI).

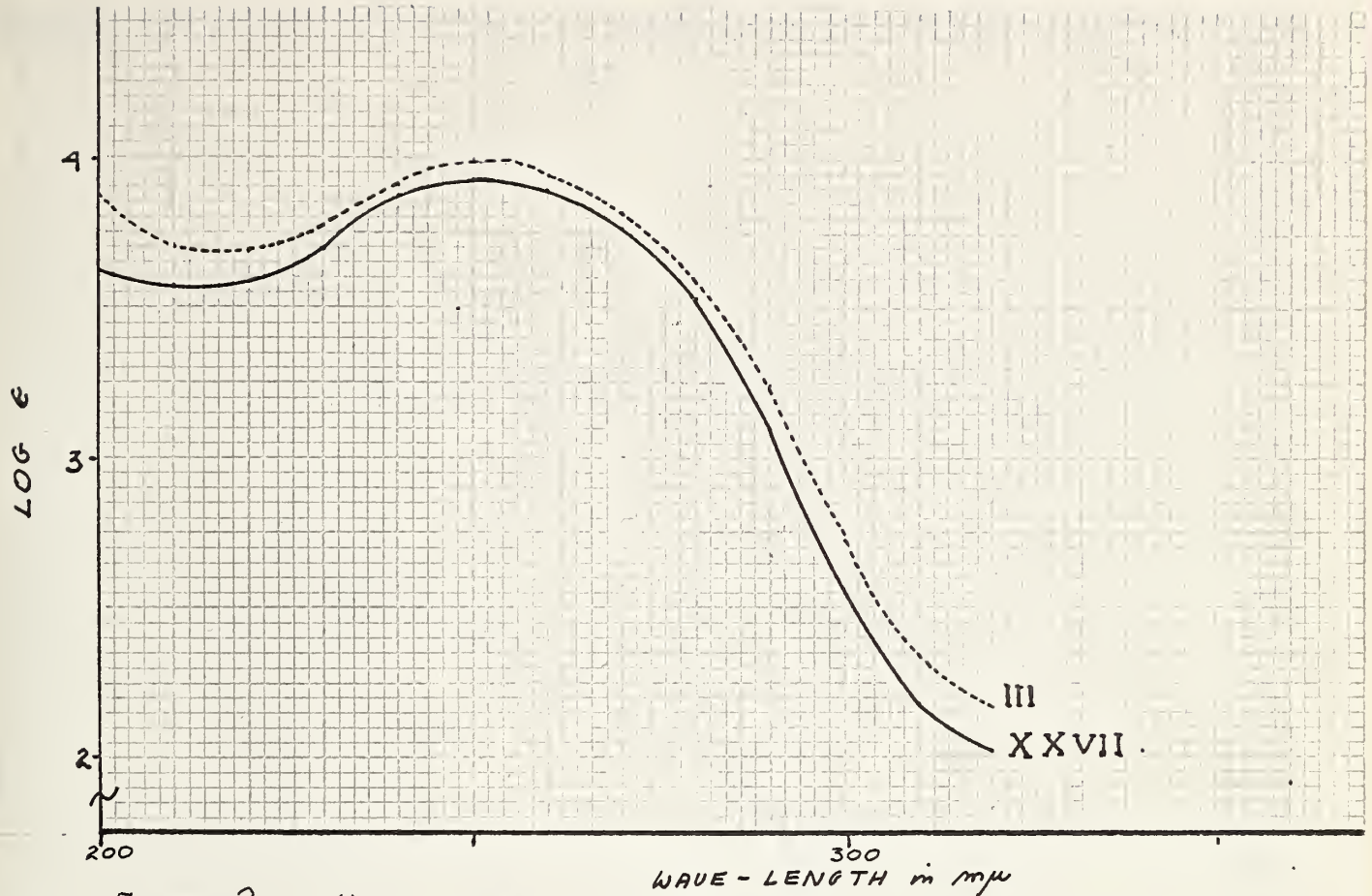


FIGURE 2A. ULTRAVIOLET SPECTRA OF 3.4.5.6.7.8- HEXAHYDROCARBOSTYRIL (XXVII) AND α-OBSCURINE (III).

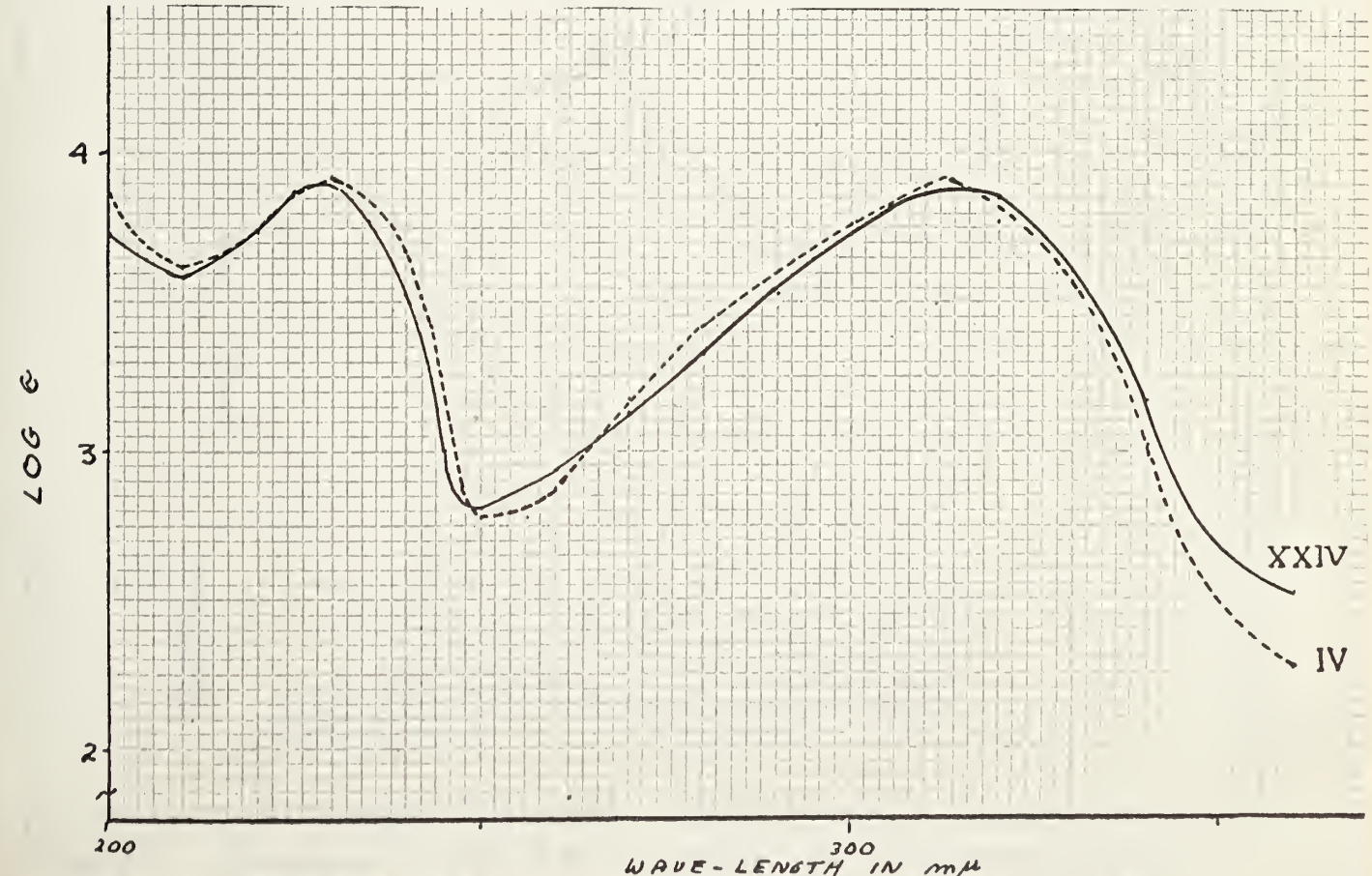


FIGURE 2B. ULTRAVIOLET SPECTRA OF ~~3.4~~.5.6.7.8- ~~HEXA~~TETRAHYDROCARBOSTYRIL (XXIV) AND β-OBSCURINE (IV).

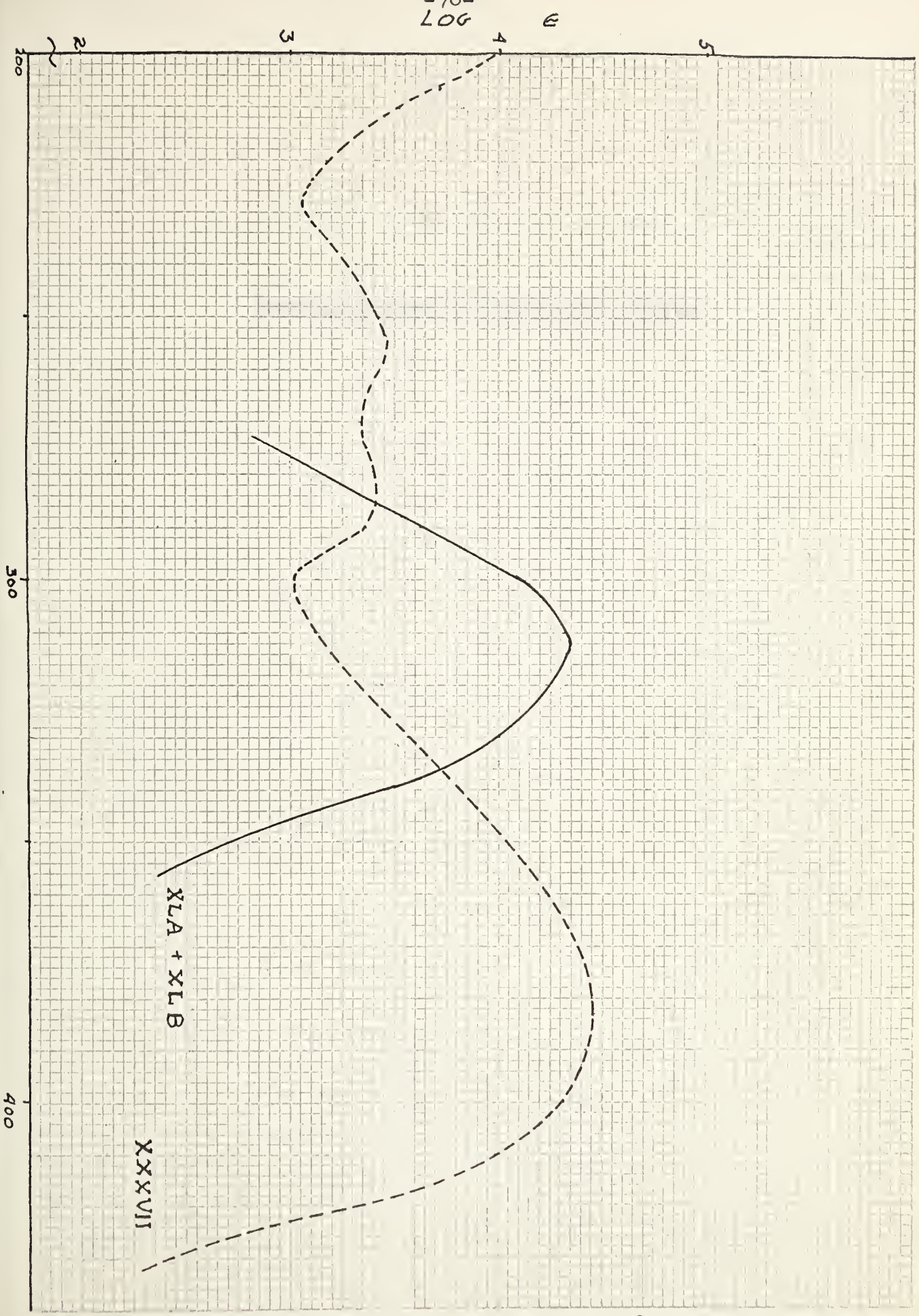


FIGURE 3. ULTRAVIOLET SPECTRA IN ETHANOL OF 2-AMINOMETHYLENE-CYCLOXEXANONE (XLA AND XLB) AND THE YELLOW COMPOUND (XXXVII).

OPTICAL ROTATORY DISPERSION CURVES

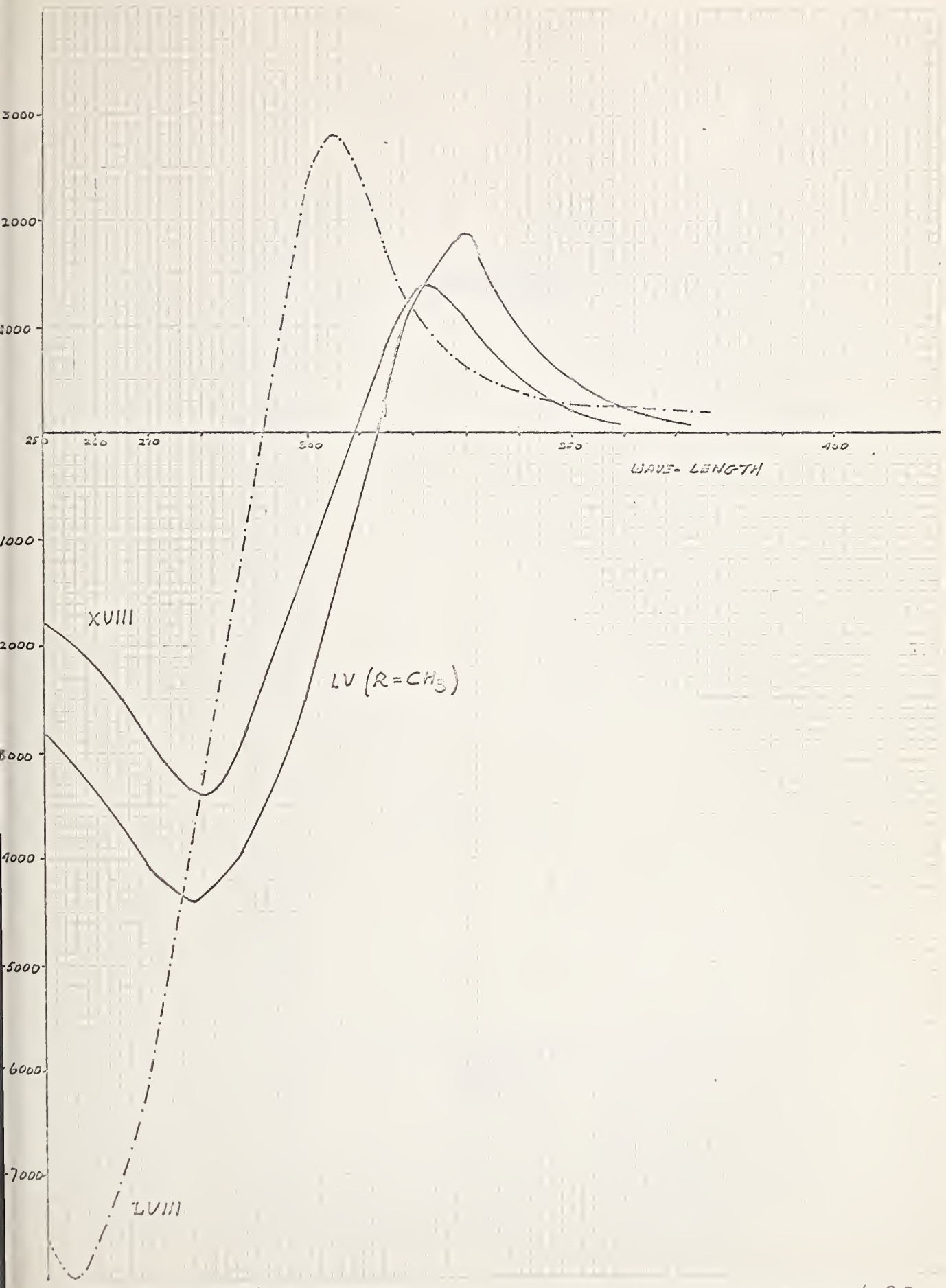


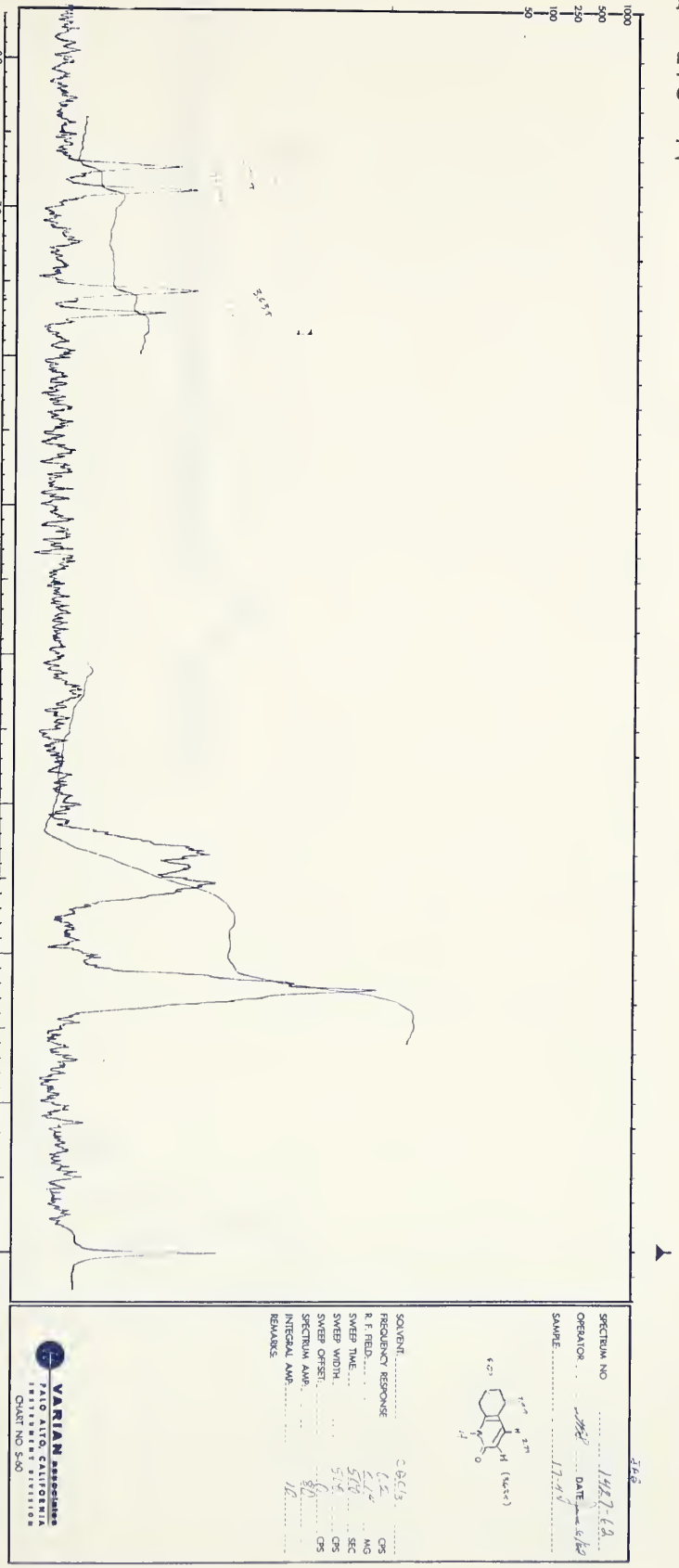
FIGURE 1. OPTICAL ROTATORY DISPERSION CURVES IN METHANOL OF ALKALOID L.2D (XVIII), α-KETOL FROM LYCOCLAVINE (LVIII), AND THE α-METHOXY COMPOUND (LV, R= CH_3)

NMR SPECTRA

NMR 1 ($CDCl_3$)

5,6,7,8-tetrahydro-carbostyril

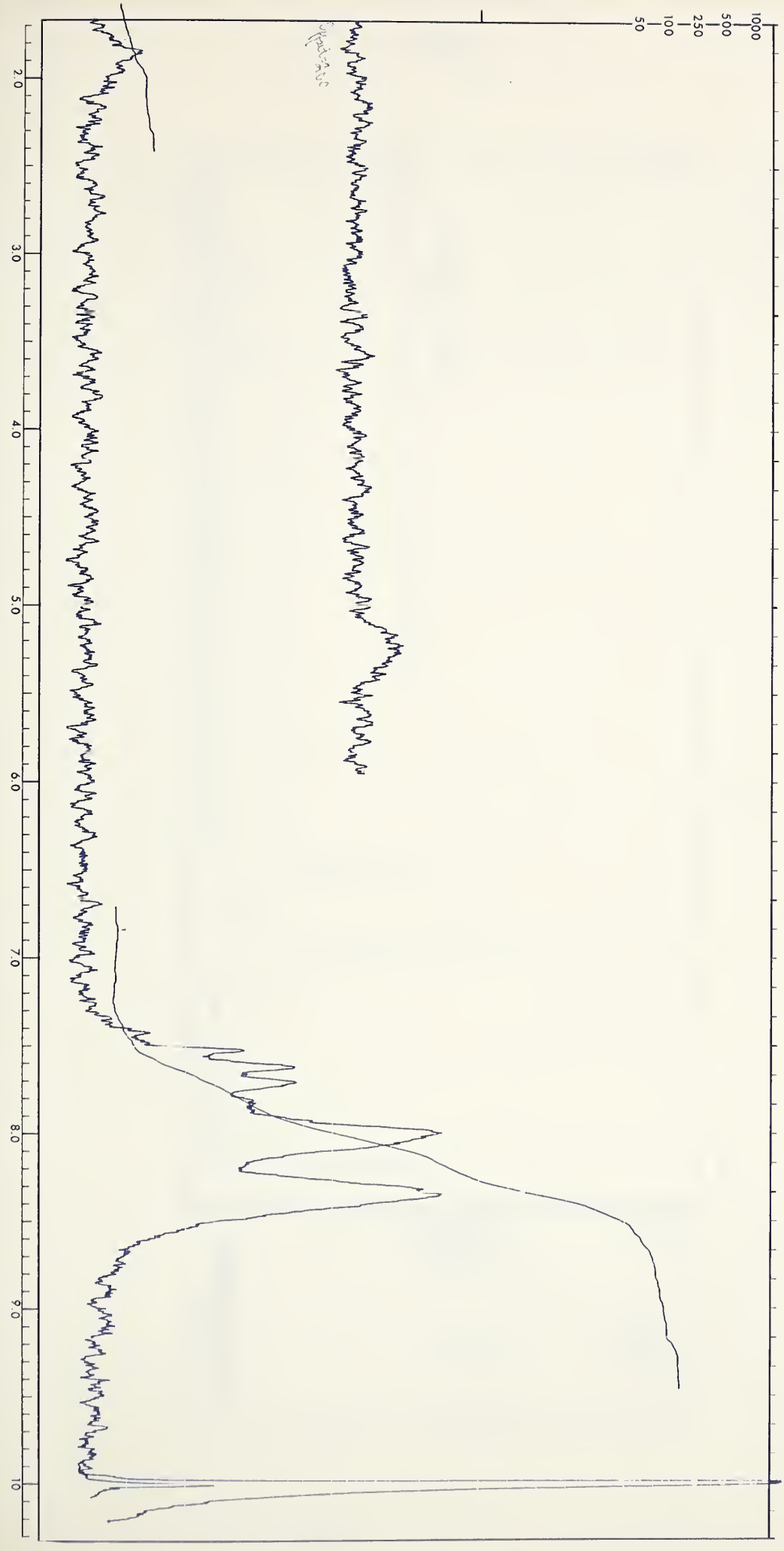
XXIV

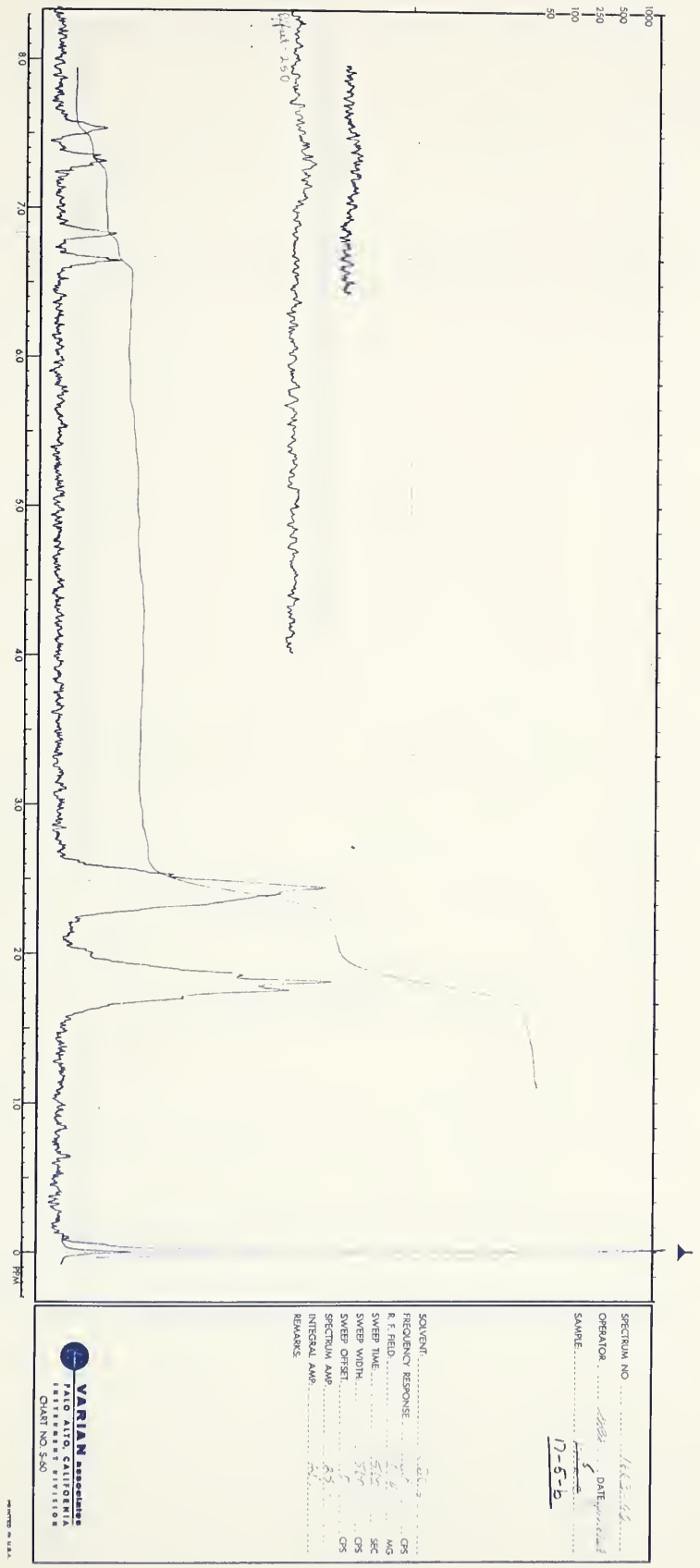


NMR 2 ($CDCl_3$)

3,4,5,6,7,8- hexahydrocarbostyril

XXVII

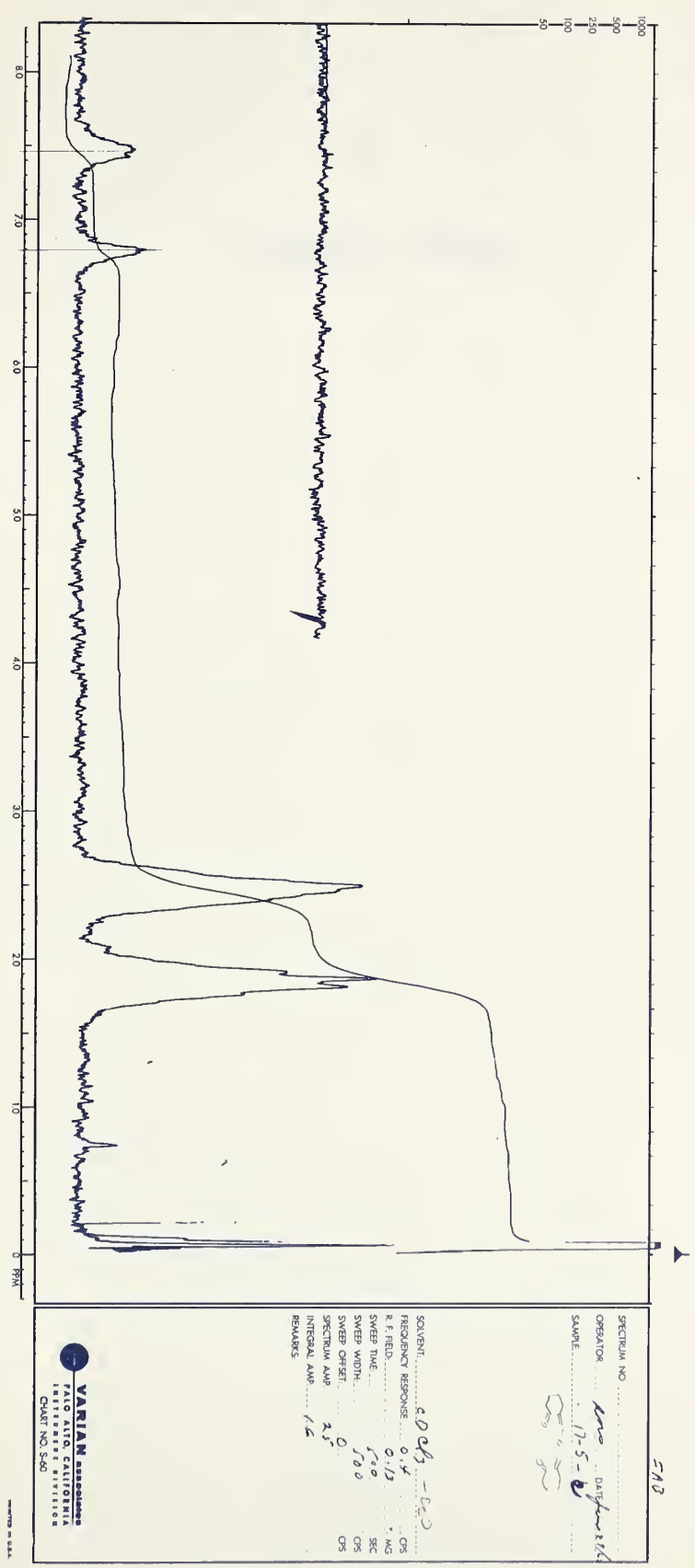




NMR 3 ($CDCl_3$)

Yellow compound

XXXVII

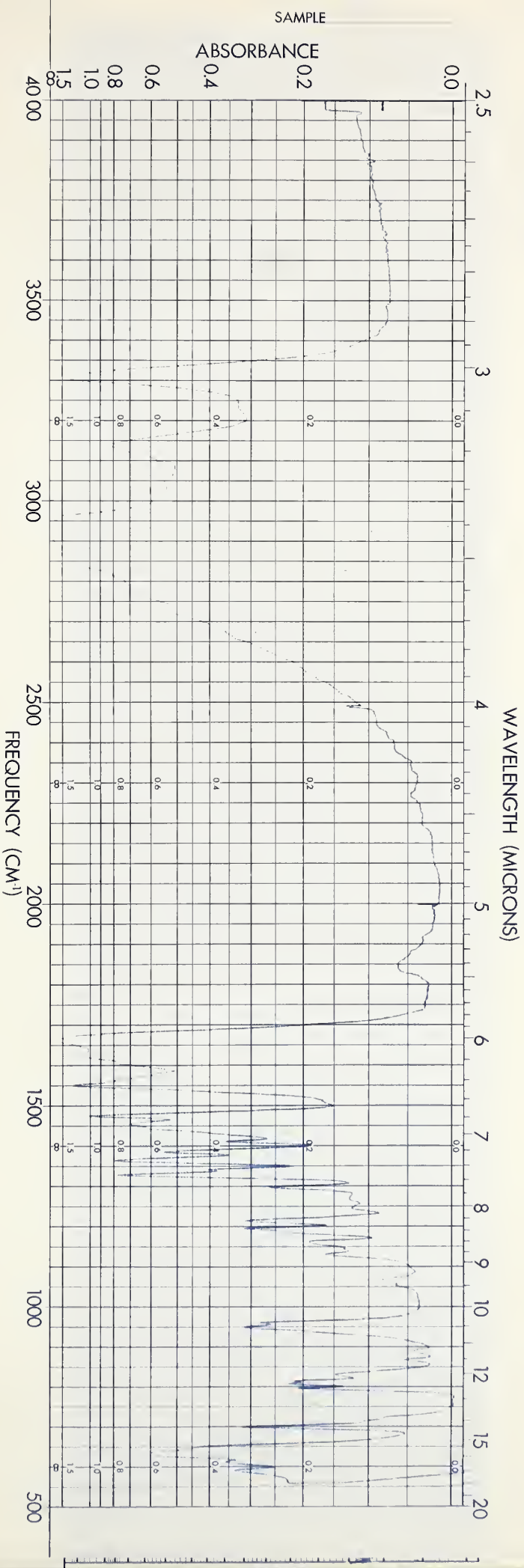


NMR 4 ($CDCl_3$+D_2O)

Yellow compound

XXXVII

INFRARED SPECTRA



I.R. 1 (nujol)

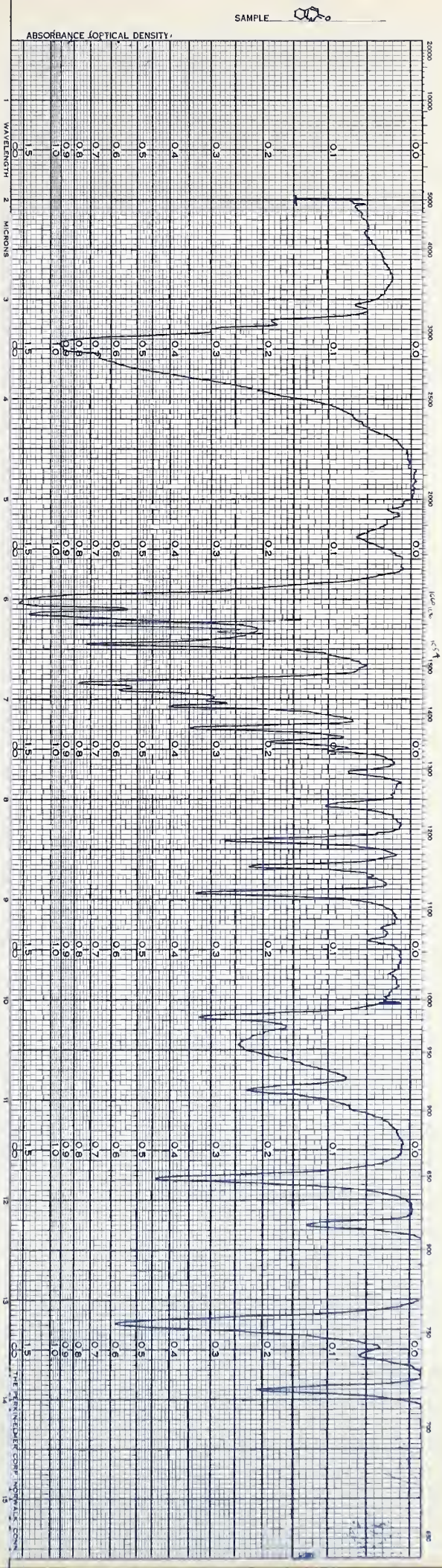
3-amido-5,6,7,8- tetrahydrocarbostyril

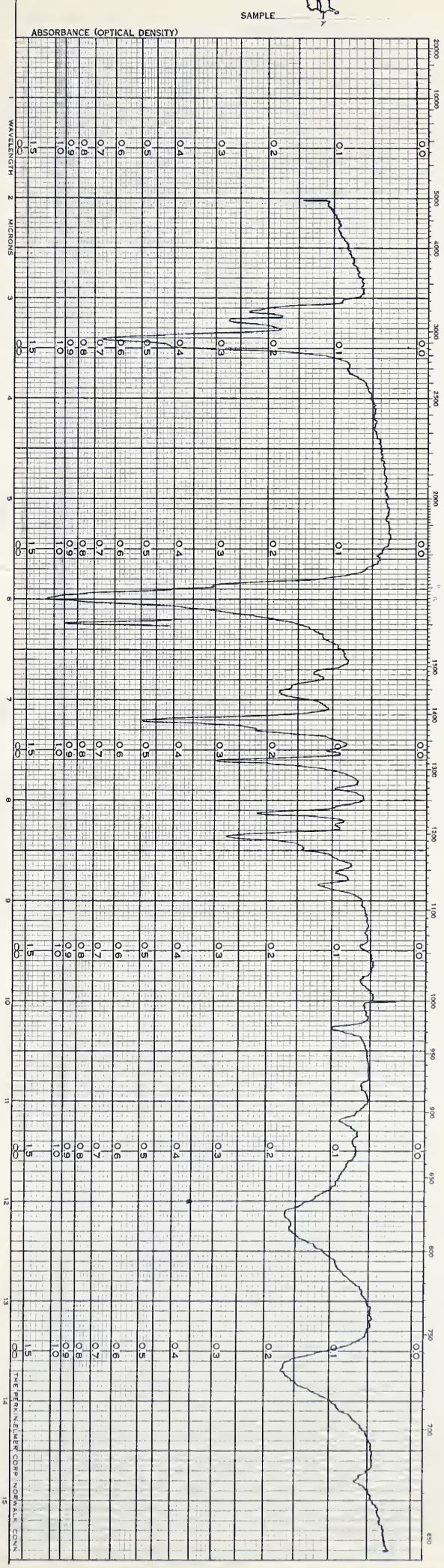
XXV

I.R. 2 (nujol)

5,6,7,8- tetrahydro-carbostyril

XXIV

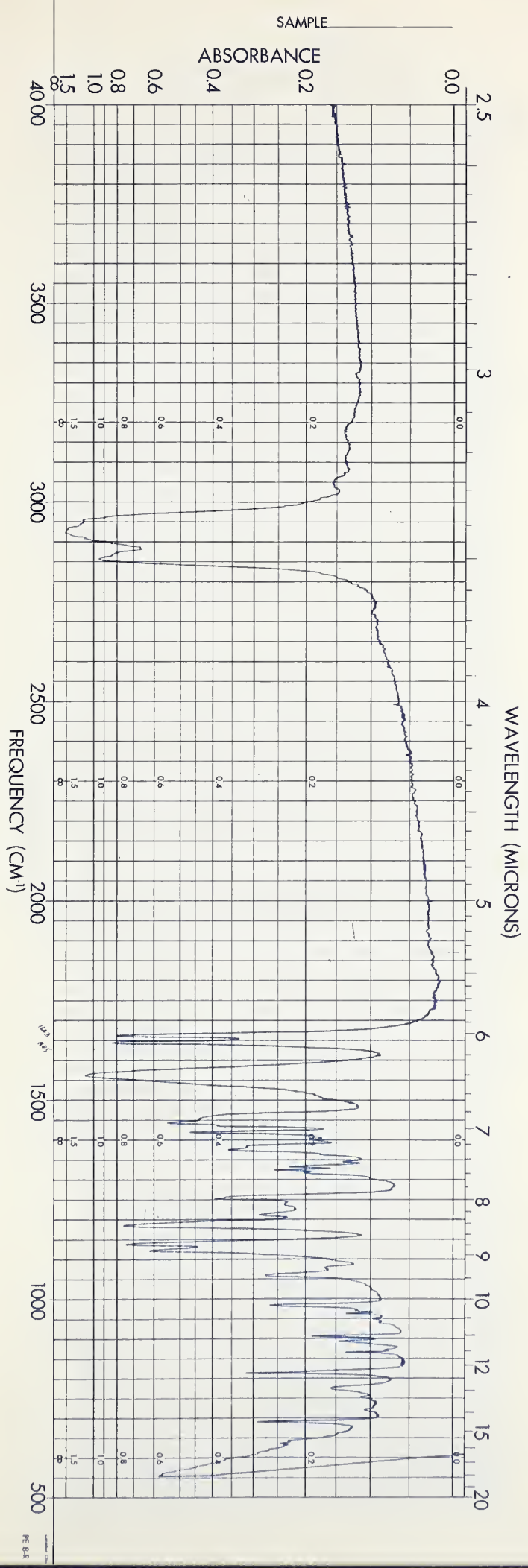




I.R. 3 (nujol)

3,4,5,6,7,8-hexa-hydrocarbostyril

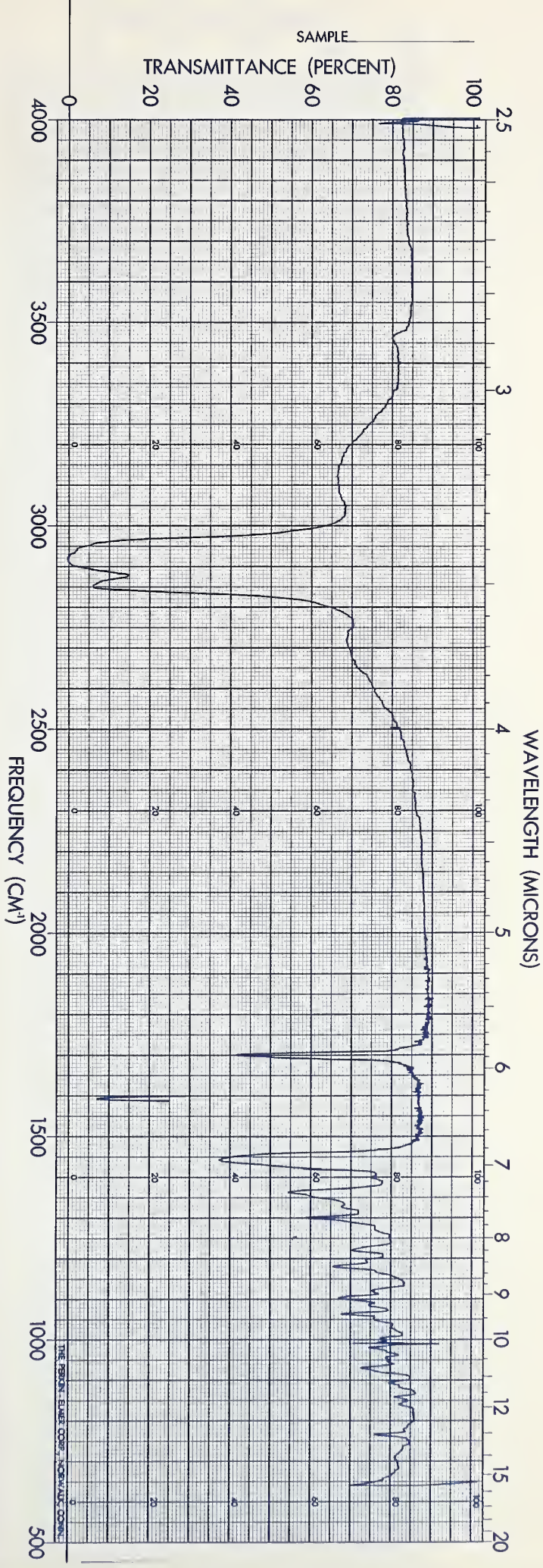
XXVII



I.R. 4 (nujol)

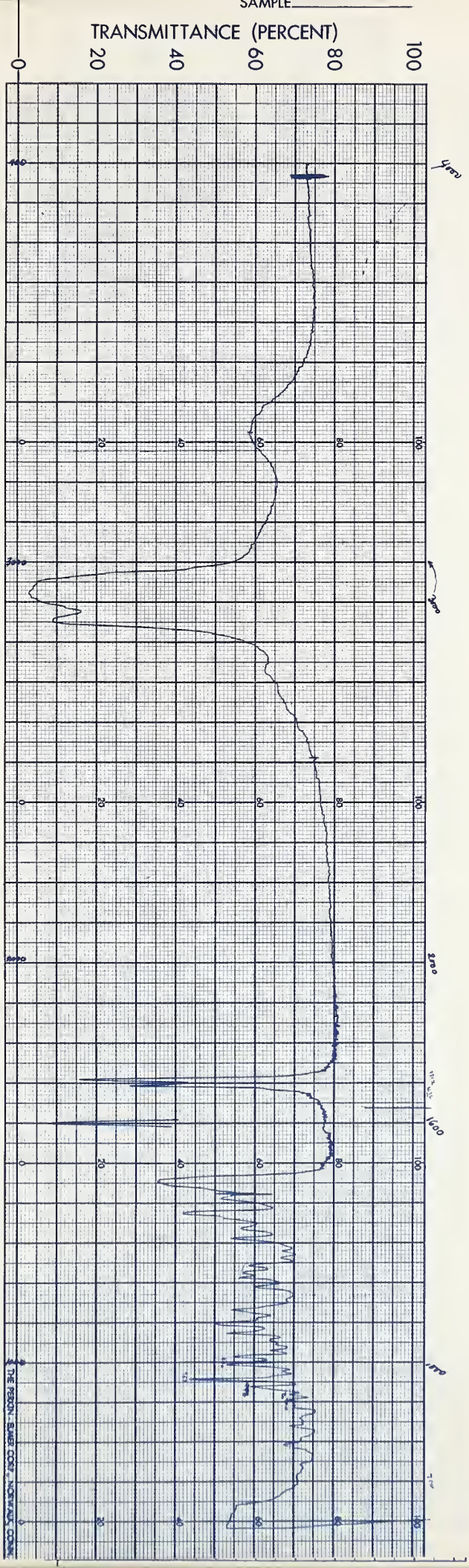
Yellow compound

XXXVII



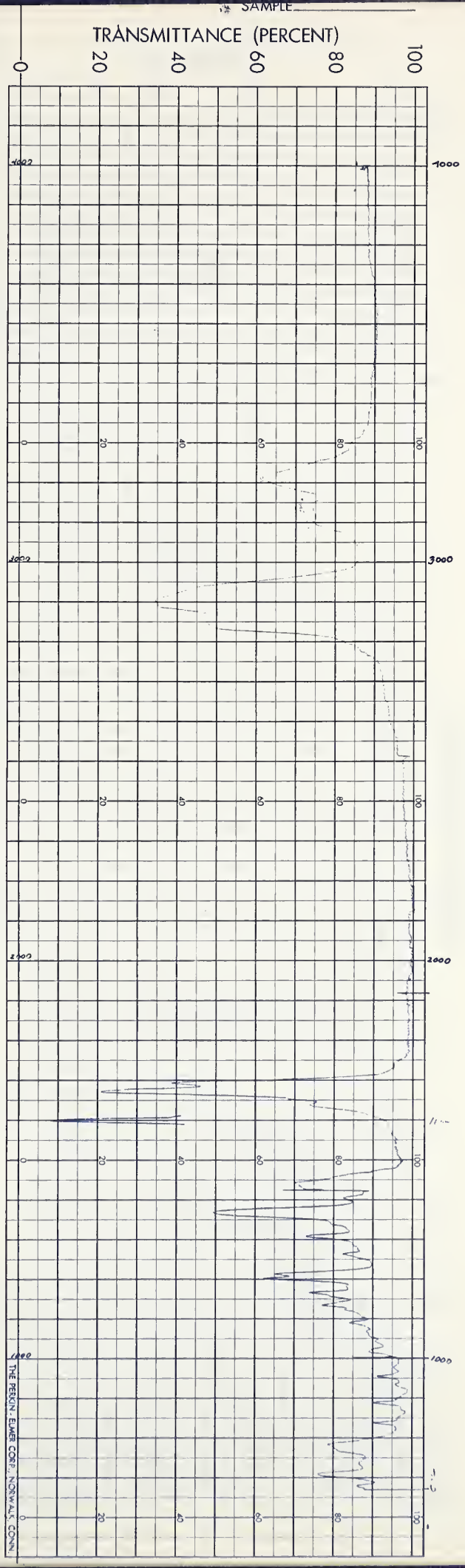
I.R. 5 (nujol)

208° complex



I.R. 6 (nujol)

180° complex



I.R. 7 (nujol)

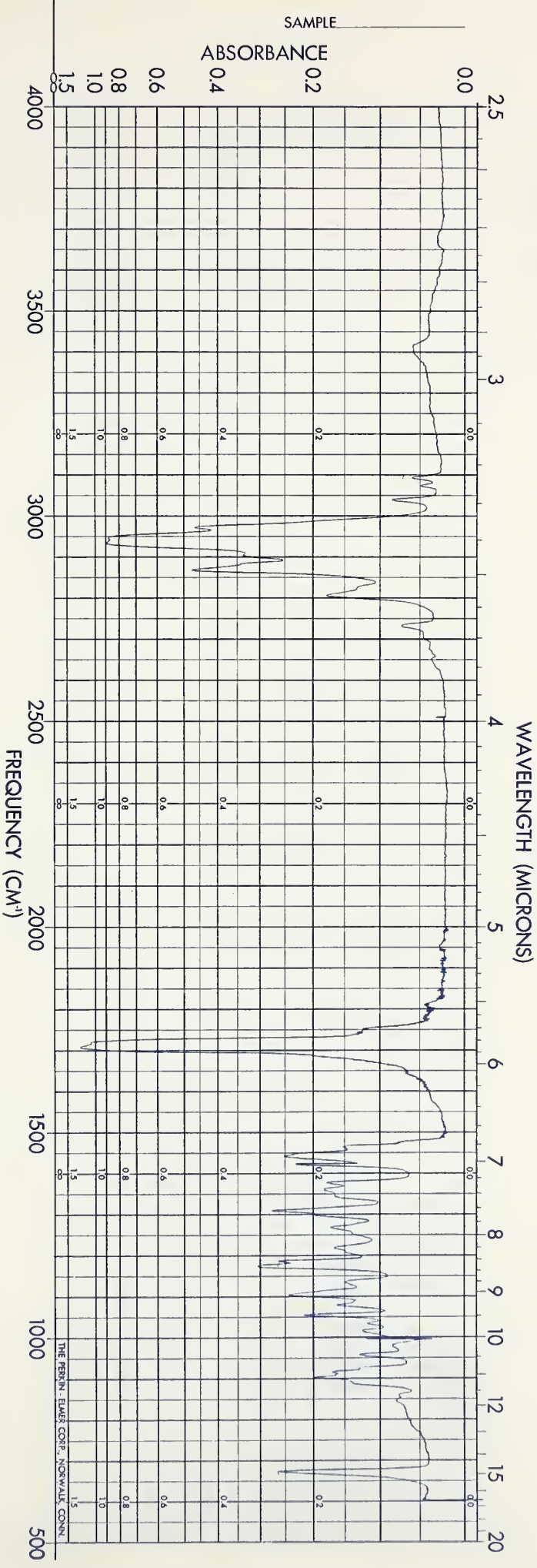
De-N-methyl-α-obscurine

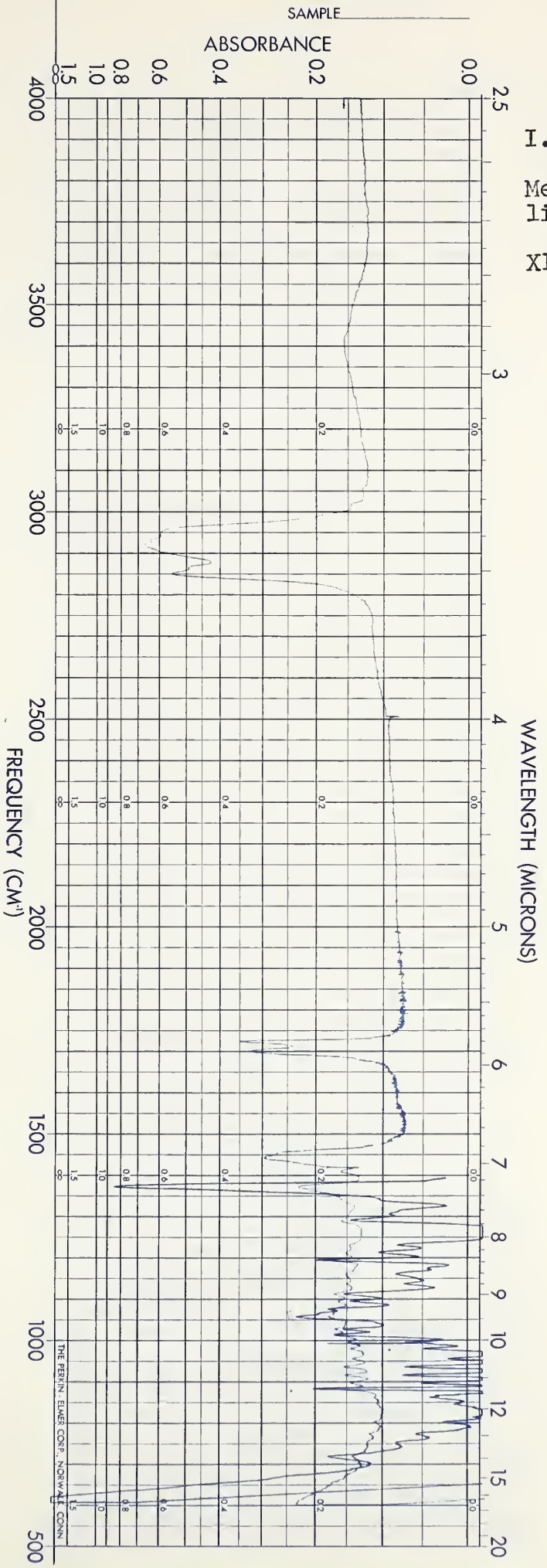
XIV

I.R. 8 (CCl_4)

Clavolonine diketone

XLVIII





I.R. 9 (nujol)

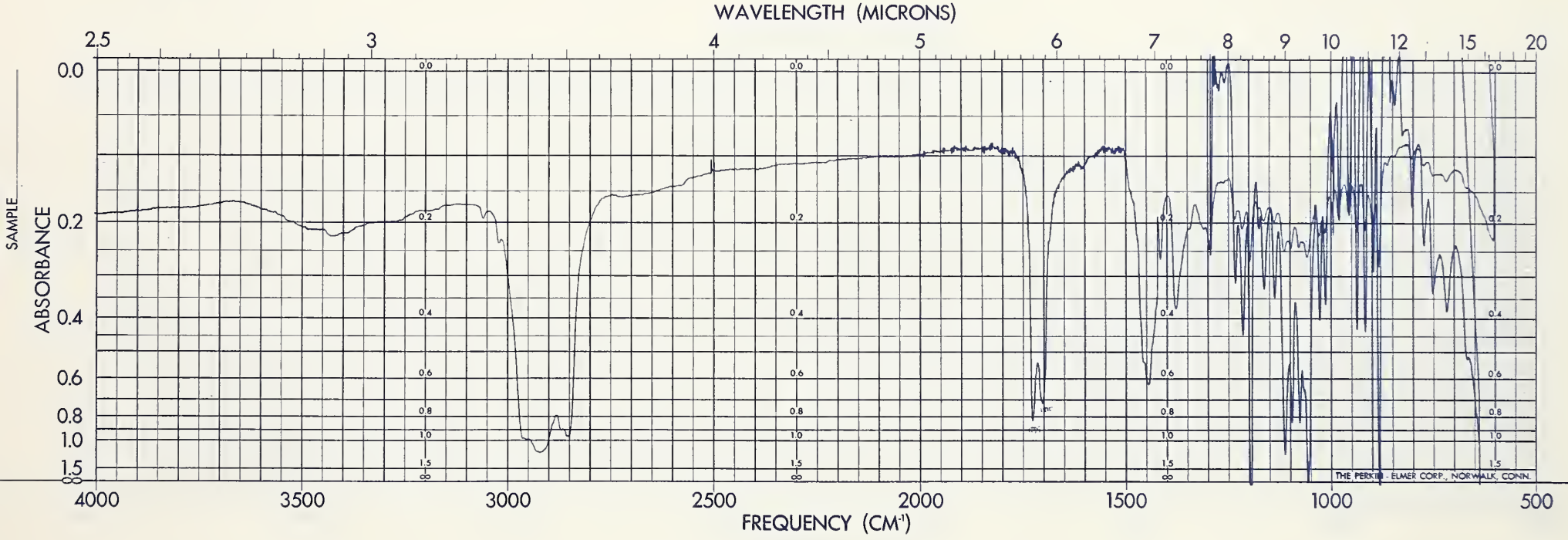
Methiodide of annofoline diketone

XLVIII

I.R. 10 (nujol)

Methiodide of clavo-
lonine diketone

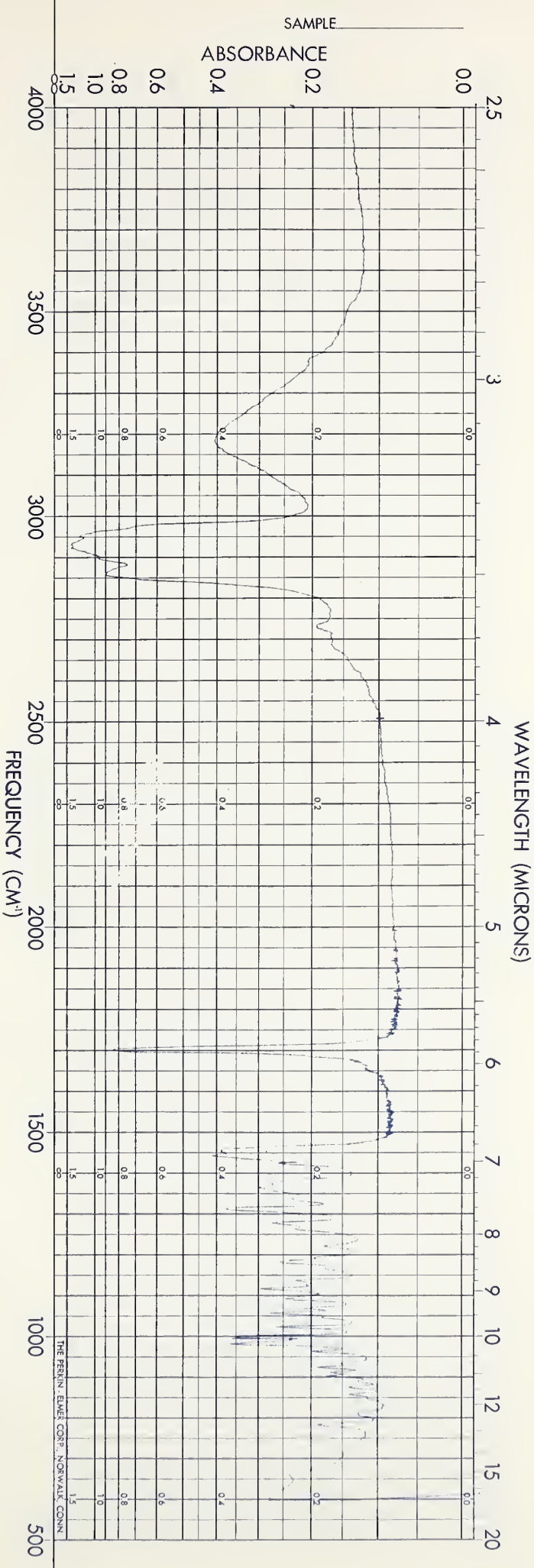
XLVIII

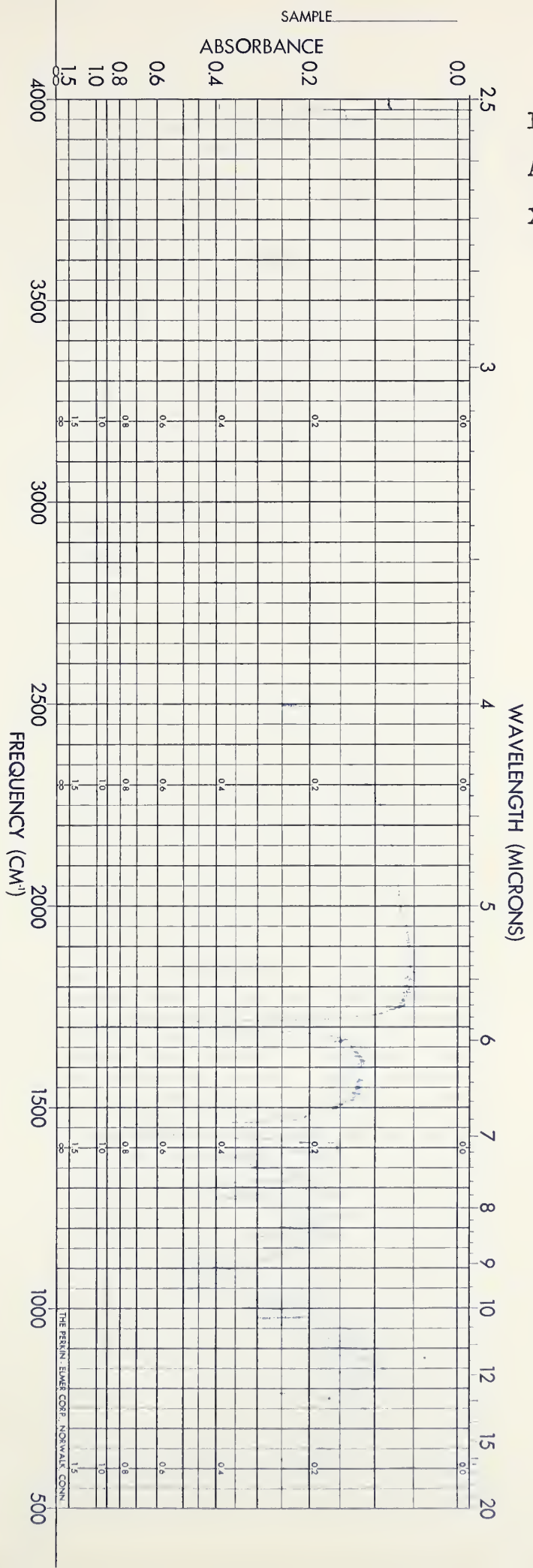


I.R. 11 (nujol)

Epiclavolonine

LV

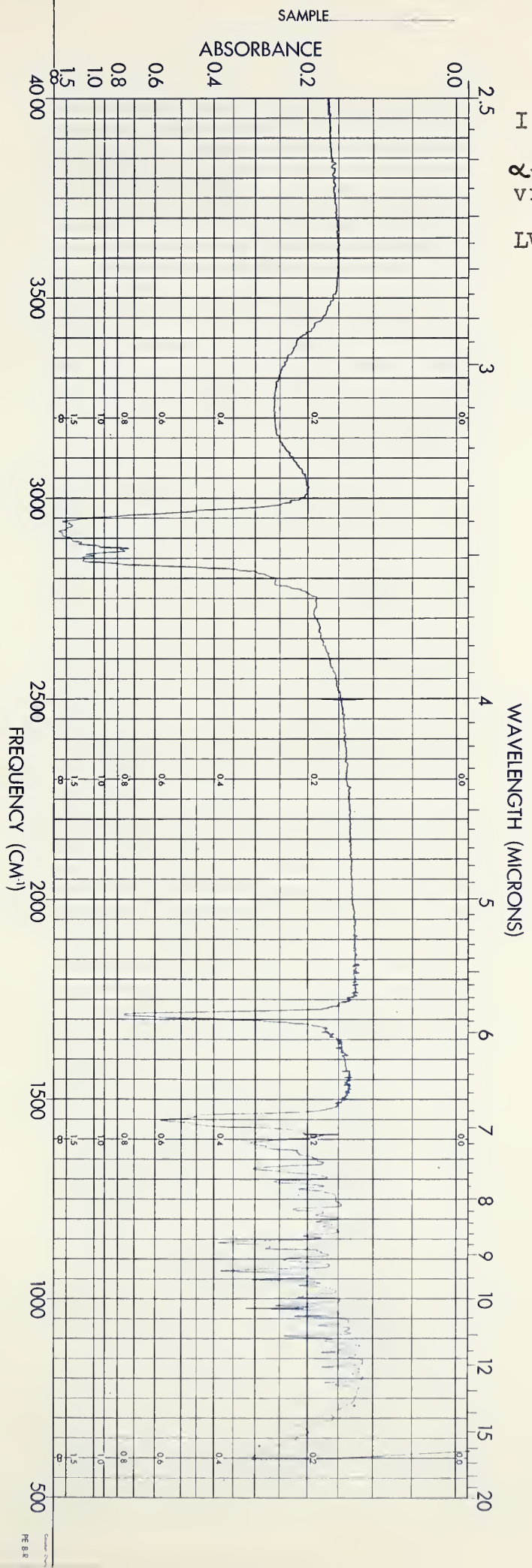




I.R. 12 (nujol)

Alkaloid L. 20

XVIII



I.R. 13 (nujol)

α-Ketol from lycoclavine

LVIII

I.R. 14 (nujol)

Methoxylycopodine

XLV (R=CH3)